2010

Water analysis for emerging environmental contaminants

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WATER ANALYSIS FOR EMERGING ENVIROMENTAL CONTAMINANTS

by

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A DISSERTATION

Presented to the Faculty of the Graduate School of the

MISSOURI UNIVERSITY OF SCIENCE & TECHNOLOGY

In Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

In

CHEMISTRY

2010

Approved by

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PUBLICATION DISSERTATION OPTION

This dissertation consists of the following five articles that have been published, submitted for publications, or will be submitted for publication as follows:

Pages 11-28 were published in JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY.

Pages 29-48 were published in ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH INTERNATIONAL.

Pages 49-68 were published in WATER SCIENCE AND TECHNOLOGY.

Pages 69-85 were submitted for publication in ANALYTICAL METHODS.

Pages 86-106 were submitted for publication in WATER RESEARCH.

ABSTRACT

The presence of emerging environmental contaminants in water bodies used either as drinking water or for recreational purpose has received considerable attention in the recent years. The emerging environmental contaminants can be defined as a wide range of chemicals that have been determined in the environment which may present serious health risks for humans. The occurrence of these contaminants indicate that both household and industrial chemicals have been introduced to water resources, a wide variety of chemicals, such as disinfection byproducts, pharmaceutical and personal care products and so on, have been detected at certain levels in either water bodies or treatment plants in worldwide. Although developments in new regulations and detection methods have taken place in the past decades that impact water analysis, there is currently no validated EPA or consensus organization methods for many of the listed emerging environmental contaminants. This body of work developed LC/MS/MS or ICP-MS based techniques for water analysis of several classes of emerging environmental contaminants, including herbicides degradation byproducts; cyanotoxins; *N*-nitrosamines and heavy metal leaching from plastic bottles. In addition, the developed methods were used to conduct high throughput screening of these emerging contaminants in water samples of various types, and to investigate the removal efficiency of these contaminants by using various oxidants and physical treatment with emphasis on analysis and treatment.

ACKNOWLEDGMENTS

The work presented in this doctoral dissertation would never have been possible without the continual support and guidance of my advisor, mentor and friend, Dr. Yinfa Ma. His direction in all aspects of my graduate work was invaluable and will be forever appreciated.

I would also like to thank my doctoral committee members, for the various ways in which they assisted me in my work. I would like to thank Dr. Craig D Adams, who is my co-advisor, for his enthusiastic patience in advising me on experimental designs and writing. I would like to thank Drs. Nam and Winiarz for advice in their respective fields of expertise. I would like to thank Dr. Whitefield for his continual support. This work was supported by funds from the Missouri S&T Department of Chemistry, the Environmental Research Center and Missouri Department of Natural Resources. I am grateful for their financial support. Sincere thanks are extended towards to Mr. Terry Timmons at Missouri Department of Natural Resources for his great help in arranging water sample collection from water treatment plants in Missouri. I want to express my special thanks to Dr. Honglan Shi for her time and assistance in operating instrumentation. I would like to thank the Department secretaries, Carol Rodman, Donna Riggs and Kathy Eudaly, who were constantly eager to help, and provided a wealth of timely information.

I would like to thank my wife, Lin Zhu, for her untiring support and encouragement. Finally, I would like to thank my parents, for giving me strength and forever holding up the highest standard, without which success would have been impossible.

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INTRODUCTION

Emerging Environmental Contaminants

The presence of emerging environmental contaminants in water bodies used either as drinking water or for recreational purpose has received considerable attentions in the recent years. The emerging environmental contaminants can be defined as a wide range of chemicals that have been determined in the environmental which may present serious health risks for humans. The occurrence of these contaminants indicate that both household and industrial chemicals have been introduced to water resources, a wide variety of chemicals, such as disinfection byproducts, pharmaceutical and personal care products and so on, have been detected at certain levels in either water bodies or treatment plants in worldwide [1, 2]. The fate of emerging environmental contaminants in the environment is determined by the processes how these contaminants were formed in water bodies which involve with a combination of physical, chemical, and biological processes such as hydrolysis, photolysis and biotransformation and so on. It's suggested that the transport processes are compound specific, however the occurrences of these emerging environmental contaminants in the environment are not only detected in locations near sources but present in relatively undeveloped areas or even a global scale [2].

Although some of the listed emerging environmental contaminants are known carcinogens, most of them were classified as probable human carcinogens based on

available scientific evidences because of limited human data from epidemiological studies. However, the interactions with components of the endocrine system enable those emerging environmental contaminants to affect hormone-driven processes.

_N_-Nitrosamines

_N_-Nitrosamines are potent mutagenic and carcinogenic compounds in humans and animal. Their existence has been confirmed in food products, cosmetic products, tobacco smoke, soil, and ground water. In recent years, _N_-nitrosamnines, mainly nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), have been found to form as water disinfection byproducts [3, 4]. The cancer potencies of these nitrosamines are considerably greater than those of trihalomethanes.

The United States Environmental Protection Agency (USEPA) Integrated Risk Information System has classified these _N_-nitrosamines into the B2 group, which indicates probable carcinogenicity to humans. In addition to NDMA, the USEPA has listed five other nitrosamines, including NDEA, _N_-nitrosomethylethylamine (NMEA), _N_-nitrosodi-_n_-propylamine (NDPA), _N_-nitrosodi-_n_-butylamine (NDBA), and _N_-nitrosopiperidine (NPIP), in the Unregulated Contaminant Monitoring Rule 2 (UCMR 2) to be monitored from 2008 to 2010.According to the USEPA, the maximum admissible concentration of these compounds in drinking water is 7 ng/L of NDMA, and 2 ng/L of NDEA, with a risk estimation of 10^{-5}. The USEPA has not yet set a regulatory maximum contaminant level for these compounds in drinking water. Water treatment via chlorination, chloramination, and chlorine dioxide of organic nitrogen-containing wastewater can produce NDMA at potentially harmful levels [5, 6]. NDMA can also

form, or be leached, during treatment of water using anion exchange resins. Further, the concentration of NDMA has been reported to reach 10 ng/L in surface waters and 20 ng/L in drinking water production wells that are under the influence of recharge water from wastewater treatment plants. Waters coming from purified sewage may be contaminated with more than 100 ng/L of NDMA [7, 8]. The concentration of nitrosoamine also increases with the concentration of monochloramine as does the reaction time. The maximum concentration of NDMA has been shown to be formed at pH 7-8, a typical level of many drinking water treatment plants.

Herbicides Degradation Byproducts

Herbicides are widely used in various combinations at many stages of cultivation and during postharvest storage. There are increasing concerns about the public health impact of herbicide degradation byproducts that may be present in water bodies used either as drinking water or for recreational purposes. Undergoing certain degradation processes, herbicides generate a complex pattern of degradation products that can be transported to ground water and streams. Aerobic microorganisms facilitate herbicide degradation in the soil, and sulfonic acid (ESA) and oxanilic acid (OA) are the two most common herbicide degradation products. Both ESA and OA degradation products of herbicides have been detected more frequently and at higher concentrations than their parent compounds in surface water and ground water [9].

These findings highlight the importance of analyzing degradation compounds of herbicides to assess the occurrence and environmental fate of herbicides in hydrologic systems. The United States Environmental Protection Agency (U.S. EPA) Office of

Drinking Water has defined drinking water quality guidelines for many parent herbicides, but guidelines for ESA and OA degradations are relatively uncommon. Only minimum reporting levels are indicated in the Unregulated Contaminant Monitoring Regulation (UCMR) published by the U.S. EPA. Studies [10] have shown that in the Midwest ESA and OA degradation products of herbicides were present in some ground water and were generally present more frequently than the parent compounds. Their results demonstrate that ESA and OA degradations have enormous potential to contaminate ground water since they are relatively mobile and persistent in soil.

Heavy Metals

The presence of hazardous metal contaminations in bottled water has raised serious public health and safety concerns in water industries. Some heavy metals, particularly Antimony, used as a catalyst during plastic syntheses are among the most important contaminations which may present serious health risks for the human population. Not only bottles for bottled water but also other plastic bottles for other drinking beverages, such as coffee, juice and milk, are associated with the metal contaminations. Concern over human exposure to metal release from plastic bottles has increased significantly in recent years [11]. In daily life, almost all types of recycling plastics are used for bottling and storage of water and other beverage as well as food. Contamination with metals leaching from plastic bottles was evaluated by many researchers [12-19]. However, previous works only focused on antimony leaching in bottled water, which were made of No.1 plastic material, upon different treatment such as heating, cooling, sunlight exposure and so on. Antimony concentration was reported at or

above the maximum allowable value [20, 21]. It was found that high temperature, long-term storage can yield antimony concentration that approach or exceed the 6ppb Maximum Contamination Level (MCL) while pH range 6-8, sunlight had small effects on antimony leaching. Thus, the possible human health impact of antimony in bottled water has become a great concern from consumers to drinks industries [22]. Little information was reported for other metal elements leaching from plastic bottles made of other different recycling materials, No.2 to No.7, upon these treatments. Because plastic bottles are used not only for drinking water, but also for other purpose, such as coffee, fruit juices, milk, and other beverages, it is very crucial to understand any factors that may affect the release of hazardous metal contaminants. For example, Orange juices, apple juice and other acidic beverages are typically in the pH range of 3-5 regardless of types of storage.

Cyanotoxins

Cyanotoxins continues to be of interest in the United States and in other countries around the world. A survey reported that 70% of these algal blooms are potentially toxic by releasing cyanotoxins [23, 24]. The presence of cyanotoxins in surface or drinking water may cause serious health risks to humans and animals. The major cyanotoxins include cylindrospermopsin(CYN), microcysitins(MCs) and saxitoxins(STXs) [25]. MCs are the most common cyanotoxins which can be produced by several cyanobacteria such as *Microcystis, Anabaena and Nostoc.* Microcysitins have been found in many countries including Australia, Canada, China, Holland, and US, and the toxin levels were reported from 0.3 to 80 μg/L. Of all the MCs, MC-LR is the most abundant and the most toxic

making up 45.5% to 99.8% of total MCs concentration in natural water [23-25]. CYN was firstly identified in the species *Cylindrospermopsis raciborskii* which have began to rapidly increase and dominate some Florida water bodies since 1997. This chemical is highly water soluble and stable to relative extremes of temperature and pH [26]. STXs are representative of a large toxin family referred to as the paralytix shellfish poisoning toxins. These toxins are identical to those produced by some toxgenic marine dinoflagellates that accumulate in shellfish that feed on those algae, It's the most powerful marine toxin currently know and among the most dangerous poisons on Earth. STX and neo-STX have been reported in freshwater cyanobacteria including *Aphanizomenon spp.* and *Lyngbya wollel* [27, 28]. cyanobacteria (blue– green algae), other freshwater algae, and their toxins have been included in the Contaminant Candidate List by US EPA.The world Health Organization recently proposed a provisional upper limit in drinking water of 1 μg/L for MC-LR.

Regulatory Status

Because surveys on the occurrence and distribution of emerging environmental contaminants is still fragmented and limited, it's not practical to include all of the emerging environmental contaminants in routine monitoring programs for the United States, however some of them have been listed under unregulated contaminant monitoring rule by US EPA. Although a few new regulatory methods have been developed in the past several years for water analysis, there is currently no validated EPA or consensus organization methods for many of the listed emerging environmental contaminants, because it is challenging for the EPA to establish regulations when

relatively limited scientific information of emerging environmental contaminants on wildlife and humans.

Analysis Trends

There is an immediate need for rapid techniques for both confirmatory and screening methods for water analysis of environmental emerging contaminants, including simple and inexpensive methods during sampling, and different types of methods are needed for different applications. Advances in analytical chemistry, instrumentation and hydrology have greatly improved our ability to identify and study emerging environmental contaminants [29].

The most sensitive technique currently used for the analysis of trace-level concentrations in water samples involves liquid chromatography-mass spectrometry, specifically LC/MS/MS, which has been widely applied in environmental analysis. Existing methods to detect N-nitrosamines in drinking water are based on solid-phase extraction (SPE) for preconcentration and analysis by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry. These methods are labor intensive, they use a large amount of organic solvents and they achieve low recoveries.

Liquid chromatography-tandem mass spectrometry (LC/MS/MS) and gas chromatography-tandem mass spectrometry (GC/MS/MS) have become the most commonly used methods for the analysis of target herbicide degradation byproducts (HDBs). To detect low-concentration HDBs, water samples are typically extracted using solid-phase extraction (SPE) before injection. However, LC/MS/MS-SPE or GC/

MS/MS-SPE are time-consuming, require high solvent volumes, and usually have low recovery rates than those methods in which no SPE is involved.

There is an immediate need for rapid techniques for both screening and confirmatory methods for the cyanotoxins analyses. A range of LC/MS/MS methods for cyanotoxins have been developed, but none have been accepted a validated US EPA methods or consensus organization methods. Most of these methods are dependent on sample cleanup methods such as solid phase extraction which require high solvent volumes and usually have low recovery rates. There is a need for simple, inexpensive methods for rapid screening of cyanotoxins in a wide variety of water types.

In this study, the LC/MS/MS or ICP-MS based techniques for water analysis are described. Approaches were developed for high throughput screening of large numbers of emerging contaminants: Herbicides Degradation byproducts; cyanotoxins; *N*-nitrosamines and heavy metal leaching from plastic bottles, and investigated removal efficiency by both chemical and physical treatment with emphasis on analysis and treatment.

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PAPER

1. Simultaneous screening of herbicide degradation byproducts in water treatment plants using high performance liquid chromatography tandem mass spectrometry

Abstract

Currently, herbicides are widely used in various combinations at many stages of cultivation and during postharvest storage. There are increasing concerns about the public health impact of herbicide degradation byproducts that may be present in water bodies used either as drinking water or for recreational purposes. This work investigated the sulfonic acid and oxanilic acid degradation products of metolachlor, alachlor, acetochlor, and propachlor in a variety of water bodies. The objective was to develop a fast, accurate, and easy method for quantitative analysis of herbicide degradation products using liquid chromatography with tandem mass spectrometry without solid phase extraction, but performing levels of detection lower than those obtained in previous studies with solid phase extraction. This research also screened 68 water samples, both untreated source water and treated water, from 34 water treatment plants in Missouri. Finally, it examined seasonal trends in levels of those degradation products by collecting and testing samples monthly. This highly sensitive method can analyze these degradation products to low ng/L levels. The method limit of quantification ranges from 0.04 to 0.05 ppb for each analyte; and quantitative analyses show a precision with RSDs of around 0.6% to 3% in treated water and 2% to 19% in untreated source water. Concentrations of alachlor ESA,

acetochlor OA, metolachlor OA, and metolachlor ESA were detected from the Missouri River and the Mississippi River water bodies in summer time. Occurrences of these compounds in treated water samples are all lower than those in the untreated source water samples

Keywords

Herbicide degradation byproduct; mass spectrometry; occurrence

Introduction

Herbicides are introduced into the environment intentionally to control certain broadleaf weed species and annual grassy weeds, barnyard grass, crabgrass, foxtails, and so on (1). They are primarily used on corn, soybean, peanuts, sorghum, potatoes, cotton, safflower, and woody ornamentals. The herbicides most commonly used in the State of Missouri include acetochlor, alachlor, propachlor and metolachlor, belonging to members of the chloroacetanilide herbicide chemical family. These herbicides were developed to be toxic to the target weed species or pests, but at certain levels they may also be harmful to humans, animals, or other organisms because they share a common mechanism of toxicity due to their ability to cause nasal turbinate tumors (2). Their high mobility in water promotes leaching from agricultural fields into ground and surface water. The transportation of herbicides in the environment depends on several factors such as application rate, rainfall, and climate (3). Herbicides in soil are subject to sorption as well

as to several biological and chemical degradation mechanisms, and they can be transported to different parts of an environment by wind, runoff erosion, and leaching. Transport by runoff and leaching may cause contamination of surface and ground water.

Undergoing certain degradation processes, herbicides generate a complex pattern of degradation products that can be transported to ground water and streams. Aerobic microorganisms facilitate herbicide degradation in the soil, and sulfonic acid (ESA) and oxanilic acid (OA) are the two most common herbicide degradation products. Barbash (4) has suggested that the transformation of metolachlor to its primary degradation product (metolachlor ESA) by soil microorganisms occurs because the chlorine atom of the parent compound is displayed by glutathione and followed by the formation of ESA degradation product after different enzymatic pathways.

Both ESA and OA degradation products of herbicides have been detected more frequently and at higher concentrations than their parent compounds in surface water (5, 6) and ground water (7). These findings highlight the importance of analyzing degradation compounds of herbicides to assess the occurrence and environmental fate of herbicides in hydrologic systems. A study of degradations in tile drain discharge from agricultural fields in central New York indicated that ESA and OA degradations can persist in agricultural soils for three or more years after application (8). A series of studies and reports (9-15) have showed that ESA and OA degradation products were more persistent and mobile than their parent compounds. These properties can lead to frequent detection and increased concentration in ground and surface water. The United States Environmental Protection Agency (U.S. EPA) Office of Drinking Water has defined drinking water quality guidelines for many parent herbicides, but guidelines for

ESA and OA degradations are relatively uncommon. Only minimum reporting levels are indicated in the Unregulated Contaminant Monitoring Regulation (UCMR) published by the U.S. EPA (16). Studies (17) have shown that in the Midwest ESA and OA degradation products of herbicides were present in some ground water and were generally present more frequently than the parent compounds. Their results demonstrate that ESA and OA degradations have enormous potential to contaminate ground water since they are relatively mobile and persistent in soil.

Liquid chromatography-tandem mass spectrometry (LC/MS/ MS) and gas chromatography-tandem mass spectrometry (GC/ MS/MS) have become the most commonly used methods for the analysis of target herbicide degradation byproducts (HDBs) (18-20). To detect low-concentration HDBs, water samples are typically extracted using solid-phase extraction (SPE) before injection (21, 22). However, both LC/MS/MS-SPE and GC/ MS/MS-SPE are time-consuming, require high solvent volumes, and usually have low recovery rates than those methods in which no SPE is involved. The objective of the present study was to develop a fast, accurate, and easy method for quantitative analysis of herbicide degradation byproducts using LC/MS/ MS, but performing levels of detection lower than those obtained in previous studies with SPE. This research also screened 68 water samples, both untreated source water and treated water, from 34 water treatment plants in Missouri during both winter and summer. Finally, seasonal trends were examined in levels of those byproducts by collecting and testing samples monthly.

Experimental

General Reagents

All chemicals and reagents used in this study were analytical grade or better unless otherwise stated. ESA and OA degradations of metolachlor, alachlor, acetochlor, and propachlor standards were purchased from Sigma-Aldrich (St. Louis, MO). Stock solutions were prepared with methanol, and solutions of other concentrations were prepared by diluting with Milli-Q water produced with a Millipore Simplicity 185 water system (Billerica, MA). Butachlor ESA (Sigma-Aldrich, St. Louis, MO) was used as an internal standard (IS).

Standard Solutions and Quality-Control Samples

Stock solution of all HDB standards were prepared at a concentration of 10 μg/mL in Milli-Q water, and working solutions were made up at concentrations in the range from 0.1 to 500 μg/L. All standard solutions were stored at -20 ^{O}C until required, and all were stable for a minimum of 3 months. Samples used for calibration and quality-control purposes were freshly prepared prior to analysis.

LC/MS/MS Analysis

Table 1 shows studied compounds, molecular mass, and minimum reporting levels (MRL). Analysis of HDBs was performed using a triple quadrupole mass spectrometer (API 4000Q TRAP) equipped with an Agilent 1100 series LC system composed of a 1100 series pump and autosampler. An automated switching valve was

used between the HPLC and mass spectrometer (MS) to direct the mobile phase to the waste or MS. Amber glass sampler vials were used for all samples. The tubing used is PEEK material. The analytical column was an Agilent Hypersil ODS (2.0x125 mm 5 μm). The elution flow rate was 300 $\mu L \cdot min^{-1}$, and the injection volume was 10 μL. Both the autosampler and column were kept at room temperature (~25 °C). Separation was achieved by a gradient elution programmed as follows: 10% B for 1 min; increased to 25% B over 3 min and maintained for 6 min; then decreased to 20% B over 0.1 min and maintained for 2 min; increased to 55% B over 9 min, increased to 95% B over 0.5 min, decreased to 92% B over 1.5 min, decreased to 10% B over 0.1 min and equilibrated at 10% B for 7 min, prior to the next injection, the total running time was 30 min. Analyst 1.4 software was used to control the LC/MS/MS systems and for data analysis. Negative electrospray ionization combined with the multiple reaction monitoring (MRM) mode was used. To select the MS/MS parameters, standards of each HDB were injected in direct-infusion mode using a syringe pump, and the declustering potential, collision energy, and collision cell exit potential were optimized for each transition. The curtain and collision gas flows were 25 $L\ h^{-1}$ and medium level, and the ion spray voltage was operated at 3000 V with a source temperature of 450 °C. A dwell time of 120 ms was used per ion pair monitored. Nitrogen for the curtain and collision gas was generated by a Peak Scientific N2 generator. Tables 2 and Table 3 summarize the instrumental conditions and method parameters.

Table 1. Studying compounds and minimum reporting levels in UCMR by USEPA

Compound	CAS Registry Number	MW	MRL*(μg/L)
Metolachlor OA	152019-73-3	279.33	2.0
Metolachlor ESA	171118-09-5	329.42	1.0
Acetochlor OA	184992-44-4	265.30	2.0
Acetochlor ESA	187022-11-3	315.39	1.0
Alachlor OA	171262-17-2	265.30	2.0
Alachlor ESA	142363-53-9	315.39	1.0
Propachlor OA	70628-36-3	207.23	N/A
Propachlor ESA	947601-88-9	257.31	N/A
Butachlor ESA	N/A	357.45	N/A

Table 2. MS parameters for determination of HDBs and IS in MRM mode

MS parameter	Propachlor ESA	Propachlor OA	Acetochlor ESA	Acetochlor OA	Alachlor ESA	Alachlor OA	Metolachlor ESA	Metolachlor OA	Butchlor ESA
Ion transitions	256/80	206/134	314/120	264/146	264/160	314/80	328/80	278/206	356/80
Collision gas(1 h^{-1})	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Polarity	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Curtain gas(1 h^{-1})	25	25	25	25	25	25	25	25	25
Dwell time(ms)	120	120	120	120	120	120	120	120	120
Ion spay voltage(V)	-3000	-3000	-3000	-3000	-3000	-3000	-3000	-3000	-3000
Heater temperature(°C)	450	450	450	450	450	450	450	450	450
Declustering potential(V)	-100	-10	-125	-60	-55	-60	-130	-65	-95
Collision cell exit potential(V)	-5	-9	-7	-7	-5	-9	-5	-5	-13
Entrance potential(V)	-10	-10	-10	-10	-10	-10	-10	-10	-10
Collision energy(V)	-52	-12	-32	-16	-56	-18	-62	-16	-13

Table 3. LC gradient program for screening method

Time (min)	Flow rate ($\mu l\ min^{-1}$)	Eluent A H_2O, 5mM ammonium acetate	Eluent B Methanol, 5mM Ammonium acetate
0	300	90	10
1	300	90	10
4	300	75	25
10	300	75	25
10.1	300	80	20
12	300	80	20
21	300	45	55
21.5	300	5	95
23	300	8	92
23.1	300	90	10
30	300	90	10

Sampling Location and Schedule

Water samples were collected across the state of Missouri. Winter water samples were collected between February and March 2009, and summer water samples were collected between June and July 2009. A total of 68 water samples were collected from a variety of water resources, including the Missouri River, the Mississippi River, and various lake water, reservoir water, and underground wells. Both untreated source and treated water samples from each water treatment plant were analyzed. To determine whether there are seasonal trends, three river water samples were collected and analyzed monthly from February to July 2009.

Sample Collection and Storage

Water samples were collected in precleaned amber glass bottles. For tap water collection, any aerator was removed, the tap was opened, and the water was allowed to flow for about 5 min. Sample bottles were filled to just overflowing so that there was no

headspace in the bottle. For river water, a large precleaned wide mouth bottle or beaker was used to collect water at a representative area. Sample bottles were filled from the container to just overflowing, sealed and placed in a cooler with ice for overnight shipment to the lab. The samples were filtered through a 0.45 μm nylon membrane filter and stored in a refrigerator until analysis at 4 °C. The analysis was completed within a week after collection (18).

Results and Discussions

LC/MS/MS Method Validation

A total of eight HDBs were separated and detected within 30 min using this method. A representative MRM LC/MS/MS chromatogram of HDB standards in reagent water is shown in Figure 1. The first compound eluted at ~6.5 min, and the last one eluted at 24.4 min. Because alachlor OA and acetochlor OA have very similar chemical structures, it is hard to separate them at high resolution meanwhile keeping the method also working for other analytes; the same phenomenon happened for alachlor ESA and acetochlor ESA. However, the coeluting compounds can be easily differentiated by different MRM transitions and quantitations of their levels were not affected. Other HDBs were well separated chromatographically, and the peak showed very good symmetry. The precursor ion detected was the $[M-H]^-$ ion for all HDBs and the internal standard. The most abundant transition of each compound was used for quantitation. The calibration and quantification was performed on the basis of analyte/IS area ratio versus concentrations. The concentration of IS used was 5 μg/L.

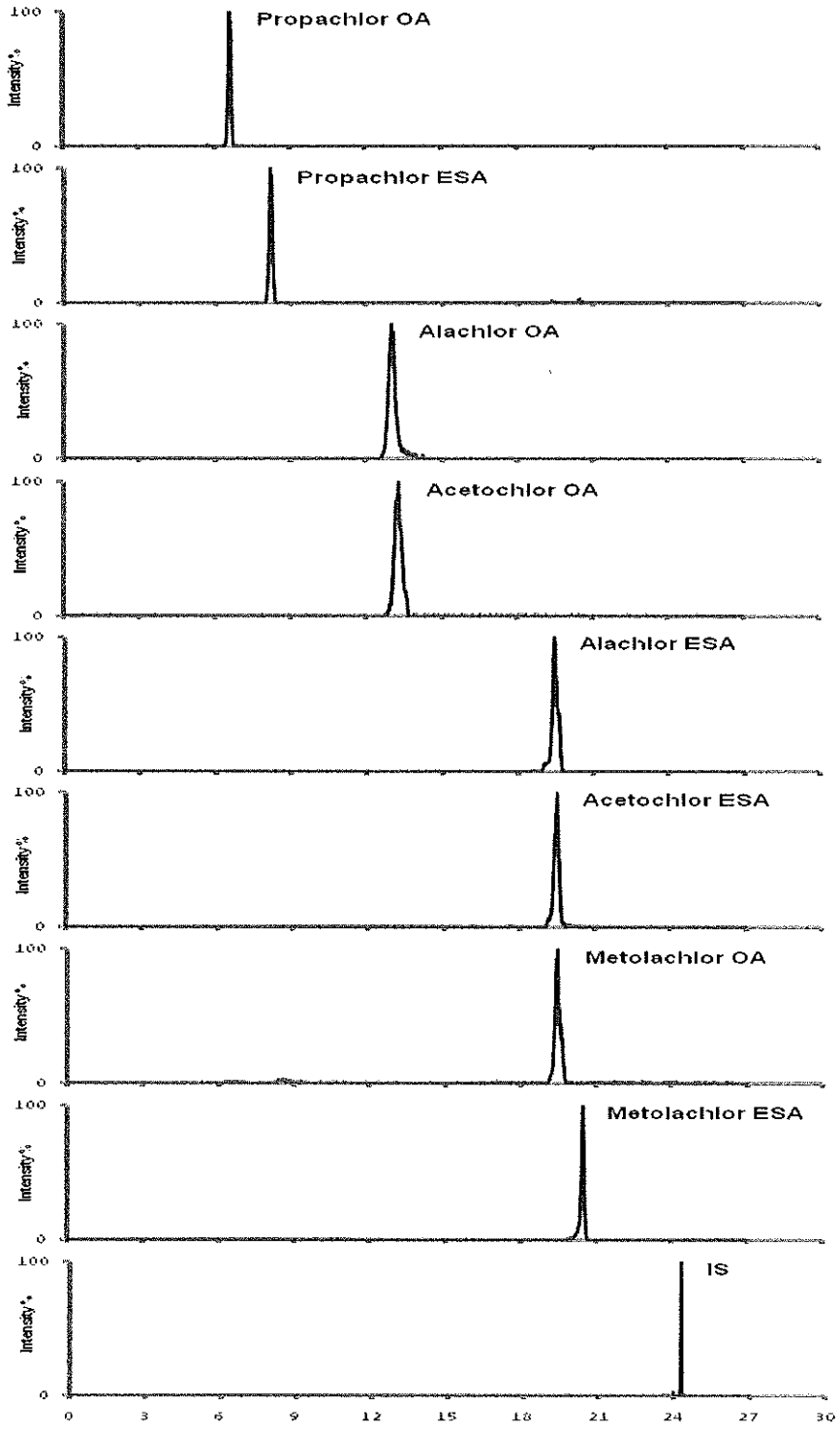

Figure 1. MRM LC/MS/MS chromatogram of HDBs in reagent water

In this study, the limit of detection (LOD) for each HDB was determined following the U.S. EPA standard method. Specifically, seven spike replicates were analyzed at a concentration of 2-5 times the estimated instrument detection limit, with LOD calculated as the product of the standard deviation(s) and Student's t (R=0.01, df=6).However ,because the instrument is sensitive and stable, this calculated LOD was too low to achieve. Thereafter, LOD for each HDB was determined as the lowest injected standard that gave a signal-to-noise (S/N) ratio between 3 and 5. The S/N ratio was calculated by measuring the peak height to averaged background noise ratio. The background noise was based on the peak-to-peak baseline near the analyte peak. The method LODs for this group of HDBs were between 0.007 and 0.009 μg/L in reagent water which were greatly improved compared with the LODs obtained in previous methods with SPE in which method LODs ranged from 0.008 to 0.043 μg/L (18). Similarly, limit of quantification (LOQ) for each HDB was obtained as the lowest injected standards that gave S/N ratio greater than 10, the method LOQ for each analyte was 0.04 or 0.05 μg/L, which are lower than those obtained by previous method with SPE in which LOQ was reported at 0.1 ppb for those studied compounds (19). A six-point standard calibration curve, in concentration ranges of 0.05-100 μg/L, exhibited good linearity.

The precision of the method was evaluated by determining the relative standard deviation (RSD) of spiked samples. The RSDs were obtained from multiple (n = 4) analyses. For analyte-free reagent water spiked with 0.1 μg/L and 10 μg/L HDBs standards, obtained from multiple (n=4) analyses. For analyte-free reagent water spiked with 0.1 μg/L and 10 μg/L HDBs standards, respectively, RSD ranged from 1.3% to 8%, with a median of 5.6%. For filtered tap water spiked with 10 μg/L HDBs standard, RSD

ranged from 23.6% to 28%, with a median of 26.1%. Figure 2 shows the MRM LC/MS/MS chromatography at a spiking concentration of 0.1 µg/L HDBs in reagent water. The validation results of the overall method are listed in Table 4.

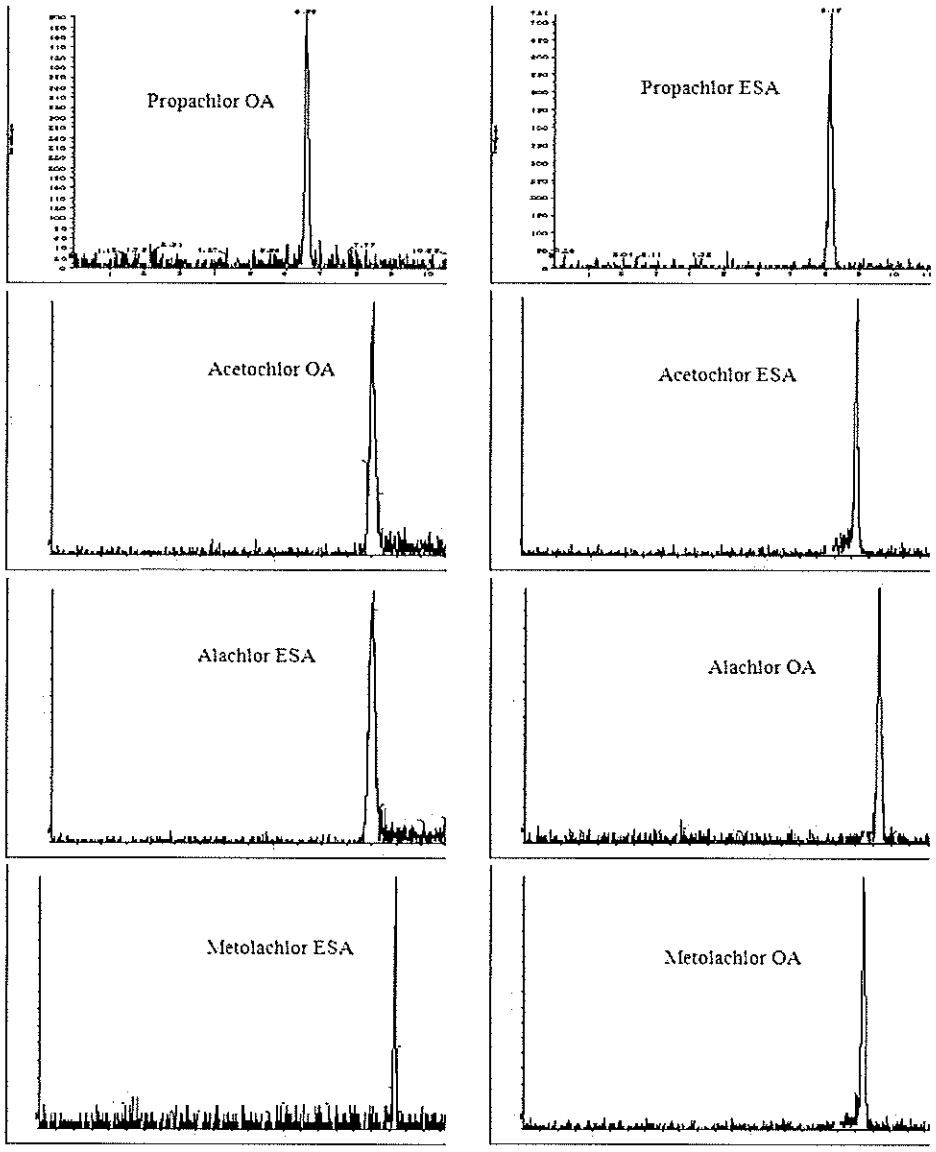

Figure 2. MRM LC/MS/MS chromatogram at a spiking concentration of 0.1µg/L in reagent water

Table 4. The validation results of overall method

Compound	LOD (μg/L)	LOQ (μg/L)	Linearity	
			Range (μg/L)	R
Acetochlor ESA	0.009	0.05	0.05~100	0.9978
Acetochlor OA	0.009	0.05	0.05~100	1
Alachlor ESA	0.007	0.04	0.05~100	0.9973
Alachlor OA	0.009	0.05	0.05~100	0.9998
Metolachlor ESA	0.007	0.04	0.05~100	0.9978
Metolachlor OA	0.009	0.05	0.05~100	1
Propachlor ESA	0.009	0.05	0.05~100	0.9995
Propachlor OA	0.007	0.04	0.05~100	0.9997

Quality Assurance/Quality Control

To ensure precision in qualitative screening, replicate of 16% of all samples were measured. For those water samples in which HDBs were not detectable, 0.1 μg/L mixture standards was spiked in and used to calculate the RSD. Analytical accuracy for the measurements was tested by spike recoveries; 16% of all samples, containing both treated and untreated source water samples, were spiked with 0.1 μg/L HDBs standards. The recoveries indicated that the matrix effects were acceptable. The QA/QC results in screening are listed in Table 5.

Table 5. QA/QC results in qualitative screening (winter and summer 2009)

Time	Water type	% RSD (n=3)	% Recovery
Feb to March	Treated	0.63~3.28	91.2~121.83
	Untreated source	1.66~4.17	95~134.1
June to July	Treated	0.67~2.41	104~121
	Untreated source	1.99~19.5	78~131

Occurrence Data in the Winter 2009

HDBs were not detected in all water samples collected in the winter. Analysis results showed that the concentrations in the water samples were all below limit of quantification for compounds of our interest. These results were expected, because HDBs are used primarily for agricultural purposes and thus applied in later winter or early spring. The water bodies most likely to contain HDBs were frozen in the winter time, and HDBs may not be transported to large rivers or reservoirs. Since no HDBs were detected in the winter season, 0.1 μg/L spiked samples were used to calculate the RSD and recovery. The QA/QC data in Table 5 assured that the data was valid.

Occurrence Data in the Summer 2009

Compared with results for winter samples, some HDBs were detected in river water samples collected in the summer 2009. The HDB concentrations detected in the water samples ranged up to 0.06 μg/L; these concentrations were much lower than those indicated in UCMR. In untreated source water samples, the Missouri River was found containing the most kinds of HDBs, including alachlor OA (0.059 μg/L), alachlor ESA (0.04 μg/L), metolachlor ESA (0.043 μg/L), and acetochlor OA (0.055 μg/L). For the water samples collected from the Mississippi River, only acetochlor OA (0.06 μg/L) and Metolachlor ESA (0.049 μg/L) were detected. Propachlor OA, propachlor ESA, metolachlor OA and acetochlor ESA were detected, but they were below limit of quantification. In treated water samples, concentrations of HDB compounds of our interest were all below limit of quantification, indicating that the current disinfection processes currently used in water treatment plants are effective to remove these

compounds. From all of the water sample analyses, the Missouri River and Mississippi River were the two major water bodies containing HDBs. Two kinds of HDBs, acetochlor OA and metolachlor ESA, were detected in untreated source water samples from both the Missouri and Mississippi Rivers. No HDBs were detected in other water sources including deep well, reservoirs and ground water. In addition, different water treatment plants, even though the source water is the same, present different HDB occurrences because of different disinfection processes used in water treatment.

Monthly Monitoring Results

To determine whether there are seasonal patterns in the occurrence of HDBs, samples from reservoir and the Missouri and Mississippi Rivers were monitored monthly from February to June 2009. Both untreated source and treated water samples were analyzed, Analysis results showed that HDBs were detected only in water samples that collected in June 2009. The HDB concentrations in the water samples that collected in other months were all below limit of quantification for the compounds of our interests.

Conclusions

This study developed a fast and easy method for HDB analysis using LC/MS/MS with no SPE. It also screened 68 water samples, both untreated source water and treated, from 34 different water treatment plants across Missouri for HDBs. Samples were collected from several water resources, including the Missouri River, the Mississippi River, ground water, lakes,

reservoirs, and wells. To study the seasonal patterns in HDB concentrations, water samples were collected and analyzed in both winter and summer. No HDBs were detected in either untreated source or treated water collected in winter (below limit of quantification). In water samples collected during the summer, concentrations of alachlor ESA, acetochlor OA, metolachlor OA, and metolachlor ESA were detected in the Missouri River and the Mississippi River. Concentrations of these compounds in treated water samples are consistently lower than those in the untreated source water samples. The seasonal monitoring data showed that alachlor ESA, acetochlor OA, metolachlor OA, and metolachlor ESA were detectable only in untreated source water samples collected in June from the Missouri River and the Mississippi River; no HDBs were detected in any water samples before summer.

Acknowledgments

We thank the Department of Chemistry and the Environmental Research Center at the Missouri University of Science and Technology, and Missouri Department of Natural Resource for financial support.

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2. Assessment of metal contaminations leaching out from recycling plastic bottles upon treatments

Abstract

Heavy metal contaminants in environment, especially in drinking water, are always of great concern due to their health impact. In this study, leaching concentrations of 16 metal elements were determined in 21 different types of plastic bottles from 5 commercial brands, which were made of recycling materials ranging from No.1 to No.7. Several sets of experiments were conducted to study the factors that could potentially affect the metal elements leaching from plastic bottles, which include cooling with frozen water, heating with boiling water, microwave, incubation with low pH water, outdoor sunlight irradiation and in-car-storage. The results revealed that heating and microwave can lead to a noticeable increase of antimony leaching relative to the controls in bottle samples A to G and some even reached to a higher level than Maximum Contamination Level (MCL) of US Environmental Protection Agency (USEPA) regulations. Incubation with low pH water, Outdoor sunlight irradiation and in-car-storage had no significant effect on antimony leaching relative to controls in bottle samples A to G, and the levels of antimony leaching detected were below 6 ppb which is the MCL of USEPA regulations. Cooling had almost no effect on antimony leaching based on our results. For other interested 15 metal elements (Al, V, Cr, Mn, Co, Ni, Cu, As, Se, Mo, Ag, Cd, Ba, Tl, Pb), no significant leaching was detected or the level was far below the MCL of USEPA regulations in all bottle samples in this study. In addition, washing procedure did contribute to the antimony leaching concentration for PET bottles. The difference of

antimony leaching concentration between washing procedure involved and no washing procedure involved (ΔC) was larger than zero for sample A to G, This interesting results showed that higher antimony concentration was detected in experiments with no washing procedures compared with those experiments with washing procedures. Our study results indicate that partial antimony leaching from PET bottles comes from contaminations on the surface of plastic during manufacture process, while major antimony leaching comes from conditional changes.

Keywords

Leaching, antimony, PET, recycling plastics

Introduction

The presence of hazardous metal contaminations in bottled water has raised serious public health and safety concerns in water industries. Some heavy metals, particularly Antimony, used as a catalyst during plastic syntheses are among the most important contaminations which may present serious health risks for the human population. Not only bottles for bottled water but also other plastic bottles for other drinking beverages, such as coffee, juice and milk, are associated with the metal contaminations. Concern over human exposure to metal release from plastic bottles has increased significantly in recent years (Kontominas et al. 2006). In daily life, almost all types of recycling plastics are used for bottling and storage of water and other beverage

as well as food. Recycling plastics No.1, Polyethylene Terephthalate (PET), is widely used for Soda bottles, water bottles and vinegar bottles (Shotyk and Krachler 2007b; Shotyk and Krachler 2007a). No.2, High Density Polyethylene (HPDE), is used for milk bottle. No.3, Polyvinyl Chloride (PVC), is used for cooking oil bottles, baby bottle nipples and coffee cups. No.4, Low Density Polyethylene (LDPE), is used for Wrapping films, grocery bags and sandwich bag. No.5, Polypropylene (PP), is used for Yogurt cups. No.6, Polystyrene (PS), is also used for coffee cups and hot beverage cups (Ahmad and Bajahlan 2007). All other types of plastics or packaging made from more than one type of plastic are labeled as No.7 which is less commonly used.

Contamination with metals leaching from plastic bottles was evaluated by many researchers (Fertmann et al. 2004; Loyo-Rosales et al. 2004; Sajiki and Yonekubo 2004; Kang et al. 2006; Kontominas et al. 2006; Mahajan et al. 2006; Momani 2006; Shotyk et al. 2006; Ahmad and Bajahlan 2007; Kale et al. 2007; Karamanis et al. 2007; Shotyk and Krachler 2007b; Shotyk and Krachler 2007a; Cao 2008; Westerhoff et al. 2008; Karamanis et al. 2009; Saeedi et al. 2009). However, previous works only focused on antimony leaching in bottled water, which were made of No.1 plastic material, upon different treatment such as heating, cooling, sunlight exposure and so on. Antimony concentration was reported at or above the maximum allowable value (Shotyk et al. 2006; Shotyk and Krachler 2007b; Shotyk and Krachler 2007a). It was found that high temperature, long-term storage can yield antimony concentration that approach or exceed the 6ppb Maximum Contamination Level (MCL) while pH range 6-8, sunlight had small effects on antimony leaching. Thus, the possible human health impact of antimony in bottled water has become a great concern from consumers to drinks industries (Suzuki et

al. 2000). Little information was reported for other metal elements leaching from plastic bottles made of other different recycling materials, No.2 to No.7, upon these treatments.

Because plastic bottles are used not only for drinking water, but also for other purpose, such as coffee, fruit juices, milk, and other beverages, it is very crucial to understand any factors that may affect the release of hazardous metal contaminants. For example, Orange juices, apple juice and other acidic beverages are typically in the pH range of 3-5 regardless of types of storage.

In this study, pH=4 was chosen to investigate the effect on metal elements leaching upon low pH treatment. It is also quite often for people to use these plastic bottles outdoor. Therefore, it is very important to understand whether nature sunlight may affect the release of hazardous metal contaminants. As we all know that temperature inside cars can exceed 45°C at the summer time in many US cities. Bottled water or other beverage storing in the car is a very common practice. Therefore, an in-car-storage experiment for 7 days was conducted. In addition, cooling, heating and microwave treatment of plastic bottles were also conducted to find out the relationships between environmental factors and metal contaminants leaching. In this paper, 21 different types of plastic bottles from 5 commercial brands, which were made of recycling materials ranging from No.1 to No.7, were selected for a comprehensive study to evaluate the effects of low pH, storage, sunlight exposure and other temperature-incubation treatments on heavy metal leaching from these commercial plastic bottles.

Different manufactures may produce different quality levels of plastic bottles which may produce different levels of heavy metal leaching even for plastic bottles made of same recycling material. Contamination stems from two possible sources. Firstly,

contaminants were produced during the manufacturing process which originally remained on the plastic surface and can be easily removed by rinsing. The other one was the residual of catalyst used in the manufacturing process. In this case, the contaminants may not be easily removed by washing but may leach out upon certain conditions, such as heating, exposing to sunlight, etc. Thus, two parallel experiments were conducted in this study. One was that all bottles were washed with Milli-Q water before each treatment; the other one was that the bottles were directly used for each treatment without washing.

Experimental

Chemicals and supplies

Twenty one different types of plastic bottles (ID# A to U) from 5 commercial brands were purchased in the summer of 2008 in the United States, which were made using recycling material No.1 – No.7, respectively. Bottles were crystal clear and had different shape for various functions. Other information was listed in Table 1. All ultrapure water used in this study was Milli-Q water using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All chemicals and reagents used in this study were analytical grade or better unless otherwise stated. Nitric acid (HNO_3) used for acidification was OPTIMA grade purchased from Fisher Scientific (Fair Lawn, NJ). Standards mixture solution was purchased from (CPI International, Santa Rosa, CA, USA). PerkinElmer Pureplus internal standard mix (Perkin Elmer SCIEX, Norwalk, CT, USA) was used as internals standard. Two types of standard reference materials (High-Purity Standards, Charleston, SC; Standard Reference material, NIST US Department of

commerce national institute of standard and technology, Gaithersburg, MD) were used as quality control in this study.

Table 1. Bottle identifications

Recycling material		Chemical name	Bottles ID
No.1	PET	Polyethylene Terephthalate	A,B,C,D,E,F,G
No.2	HDPE	High Density Polyethylene	H,I,J
No.3	PVC	Polyvinyl Chloride	K,L
No.4	LDPE	Low Density Polyethylene	M,N,O
No.5	PP	Polypropylene	P,Q
No.6	PS	Polystyrene	R
No.7	PC	Polycarbonate	S,T,U

Sample preparations

A constant volume of 20 ml ultrapure water was used in the whole experiments. Briefly, 20 ml ultrapure water was added in each bottle for each treatment. Two parallel experiments were conducted in this study. One experiment was that all bottles were washed with Milli-Q water before each treatment; another experiment was that the bottles were directly used for each treatment without washing. Detailed procedure for each treatment was described in following sections.

Boiling-water treatment experiments

Temperature incubation experiments were conducted by pouring 20 ml boiling water (100 ^{o}C) into two batches of bottles of A to U, one group of bottles were rinsed three time by ultrapure water before adding 20 ml water, the other group of bottles were directly added 20ml water without rinsing. Each bottle was covered with original cap

after temperature come down to room temperature (25°C) and stay 24 h in dark lab before analysis. Meanwhile, another set of experiments were conducted as control: similarly as procedure above, 20 ml room-temperature water was poured into two parallel groups of bottles of A to U, one group of bottles were rinsed three time by ultrapure water before adding 20 ml water, the other group of bottles were directly added 20 ml water without rinsing and stay for 24 h in dark lab before ICP-MS (Inductively coupled plasma-mass spectrometry) analysis. The dark lab was room temperature (25°C) and all bottles were covered with original cap to prevent dust or other contaminants falling into bottles.

Ice-cold water treatment experiments

Similar to the boiling-water treatment experiment, 20 ml ice-cold water was poured into two batches of bottles of A to U, one group with rinsing before adding the ice-cold water and another group was not. Then bottles were covered with original cap and were stored in dark lab for 24h before ICP-MS analysis. Control experiments were also conducted exactly same as stated in the boiling-water treatment experiments.

Microwave heating treatment experiment

Similarly, 20ml ultrapure water was added into two batches of bottles of A to U (One group with rinse and another group without rinse before adding water) and heated in a microwave (1200 Watts) for 3 mins in cook mode. After bottles were cooled down to room temperature, they were covered with original cap and stored in dark lab for 24h before ICP-MS analysis. Control experiments were also conducted exactly same as stated in boiling-water treatment experiments.

Low-pH-water treatment experiment

Briefly, two groups of bottles of A to U (one group was rinsed and another group was not rinsed before adding water) were filled with 20 ml acidic water (pH=4.0), covered with original caps and stored in dark lab for 7 days before analyzed by ICP-MS. Control experiments were also conducted exactly same as stated in boiling-water treatment experiments except that the storing time is 7 days instead of 24 h.

Outdoor sunlight-exposure experiment

Because the intensity of sunlight was variable during a day, it's very difficult to control this parameter. We conducted a 7-day exposure test and it was sunny during the whole week. As it was summer time, the sunlight was very intensive from 10:00 am to 3:00 pm in these seven days and there was no rain during nights. Two groups of bottles of A to U were filled with 20 ml water and covered with original caps. They were stored out-door and exposed directly to nature sunlight for 7 days. For the control experiments, two groups of bottles of A to U was filled with 20 ml room-temperature water (one group was rinsed and another was not rinsed before adding water) and stored in the same place as those bottles for sunlight exposure for 7 days. However each bottle in control experiment was fully wrapped with aluminum foil to prevent sunlight irradiation. All samples were analyzed by ICP-MS method after 7 days treatment.

In-car storage experiment

In this experiment, we were trying to mimic a real condition for in-car-storage. Two groups of bottles of A to U were filled with 20ml water and covered with original

caps (one group was rinsed and another group was not rinsed). All Bottles were placed on the back seats in author's car for 7 days. Author drove 5miles everyday day with windows fully closed and air conditioning off, and parked the car in an open parking lot when car was not moved. Temperature inside the car was measured three times a day. Control experiments were also conducted exactly same as stated in boiling-water treatment experiments except that the storing time is 7 days instead of 24h. All samples were analyzed by ICP-MS method after 7 day treatment.

Metal elements analysis by ICP-MS

Trace elements were analyzed by following USEPA method 200.8 (USEPA, 1994). An Elan-DRCe ICP-MS instrument (Perkin-Elmer SCIEX, Concord, Ontario, Canada) was used to perform this analysis. Table 2 lists the ICP-MS instrumental conditions and method parameters. Internal standards were added continuously online as a mixture.

Table 2. ICP-MS instrumental conditions and method parameters

Parameter	Operation setting
ICP RF power	1500 W
Plasma gas flow	15 L/min
Auxiliary gas flow	1.20 L/min
Nebulizer gas flow	1.01 L/min
Sample introduction system	Cyclonic spray chamber with Meinhard nebulizer
Detector mode	Pulse
AutoLens	Enabled
Lens voltage	6.5V
Analog stage voltage	-1600 V
Pulse stage voltage	850 V
Sampler cone	Platinum, 1.1 mm orifice
Skimmer cone	Platinum, 0.9 mm orifice
Mass resolution	0.7 amu
Operating vacuum pressure	6×10^{-6} torr
Number of replicates	3

Results and Discussions

Quality assurance/quality control (QA/QC) results

Six-point standard calibration curve for each element was conducted with this method. The method detection limit for each element was in the range from 0.001 to 0.02 μg/L with S/N ranging from 3 to 5.The limit of quantification for each element was 0.1 μg/L and the response was linear up to more than 1000μg/L (R^2>0.9999). Please note that samples were analyzed by ICP-MS right after each treatment was finished, not a single run for all samples from all treatments. Six treatments were investigated in this study which means six batches of sample runs were conducted independently. To assure the method precision and data accuracy, 10% of samples were duplicated and spike recoveries were tested in each batch of samples, Thus six sets of %STD(n=2) and spike recoveries were obtained for each element and the ranges were shown in Table 3. Analytical accuracy for the measurements was conducted using two types of reference standard materials and matrix spike recoveries for different levels of analytes. The QA/QC results were also listed in Table 3.

Table 3. Method validation and quality control results

	Detection Limit		Precision & Accuracy		Quality Control			
					Reference Std-1		Reference Std-2	
Element	LOD (µg/L)	S/N	% Spike Recovery	%STD (duplicate)	Certified value (µg/L)	%Accuracy	Certified value (µg/L)	%Accuracy
Al	0.02	3.5:1	99.4~110	0.529~2.88	125	96.1	5	93.1
V	0.02	3:1	97.5~115	0.758~3.94	35	92.8	1.2	90.2
Cr	0.02	3:1	97.6~113	0.051~3.01	20	93.8	4	101
Mn	0.02	3.5:1	94.2~112	0.231~2.48	40	95.4	12	94.5
Co	0.02	3.5:1	96.5~113	0.094~1.84	25	94.3	2	92.6
Ni	0.02	3:1	93.4~109	0.348~2.73	60	96.6	2.8	97.2
Cu	0.02	3.5:1	92.2~107	0.533~2.41	20	98.5	9	99.5
As	0.01	4:1	97.3~123	0.858~2.69	55	88.2	2.5	98.1
Se	0.02	3:1	97.4~128	1.47~6.42	11	83.9	2	98.5
Mo	0.02	3:1	100.6~111	1.03~2.63	110	94.0	5	95.9
Ag	0.02	4:1	100.5~111	0.516~2.63	2	94.3	0.8	101
Cd	0.001	3:1	87.5~108	0.031~2.35	10	99.0	2	91.3
Sb	0.02	3:1	93.0~116	1.03~2.79	55	91.5	1.5	112
Ba	0.02	5:1	91.6~110	0.292~2.93	500	96.5	15	96.7
Tl	0.001	3:1	90.5~109	0.031~2.05	10	99.0	0.009	112
Pb	0.02	5:1	96.8~116	0.377~2.77	20	92.6	3	103

Effects of cooling, boiling and microwaving on the levels of metal leaching

Several sets of experiments were conducted to study the factors that could potentially affect the metal leaching from recycling plastic bottles. Table 4 and Table 5 summarized the results testing bottles A to G filled with ice cold water. It's worth mentioning that data in Table 4 and Table 5 is normalized data which means control data was already deducted from the sample data for each bottle in each treatment. As clearly indicated by the results in Table 4 and Table 5, freezing water has no effect on the levels of metal leaching and no significant leaching was observed for all metals in our study.

Table 4. Regulated MCL of each element and metal leaching concentration ranges (μg/L) after each treatment for all bottle samples

	MCL(ppb)	Bottles A-U	
		Ranges of Normalized data	Ranges of Raw data
Sb	6	0.001~10.51	0.002~11.42
Al	200	0.001~18.08	0.002~18.21
V	N/A	0.001~0.622	0.002~1.451
Cr	100	0.002~0.65	0.003~0.843
Mn	50	0.001~1.652	0.002~1.678
Co	N/A	0.001~0.292	0.002~0.374
Ni	100	0.002~3.826	0.003~4.052
Cu	1000	0.008~4.586	0.009~5.477
As	10	0.001~0.052	0.002~0.059
Se	50	0.009-0.143	0.01-0.162
Mo	N/A	0.001-0.272	0.002-0.437
Ag	100	0.001-0.18	0.002-0.221
Cd	5	0.001~0.115	0.002~0.123
Ba	2000	0.006~7.515	0.007~7.633
Tl	2	0.001~0.281	0.002~0.288
Pb	15	0.001~4.532	0.002~4.564

Heating with high temperature can lead to faster leaching of antimony and it has been reported that antimony concentration can go up more than twice of the MCL of EPA regulation after 7 days at 80°C (Shotyk et al. 2006; Shotyk and Krachler 2007b; Shotyk and Krachler 2007a). The results of our study were also shown in Table 5. The levels of antimony concentration increased 4 times for the bottles treated with boiling water (from 2.077 to 8.145 ppb) relative to the control in bottles A to G. This level was higher than 6 μg/L which is the MCL for USEPA regulations. For bottle C, the level of antimony reached to 8.145 μg/L after the boiling water treatment, which is 33% higher than the USEPA MCL level. No significant leaching was detected for other metals in all bottles and there concentrations were way below the MCL of USEPA regulations. It can be seen that the non-washed bottles have higher levels of antimony than those of washed ones for bottles A to G.

Table 5. Antimony concentrations (μg/L) after each treatment. The upper table shows data generated from experiments in which bottles were washed before treatment. The lower table shows data generated from experiments in which bottles were not washed and directly used for treatment. Data in Table 5 is normalized data which means control data was already deducted from the sample data for each bottle in each treatment

Treatment	PET							HDPE			PVC		LDPE			PP		PS	PC		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
Cooling	0.675	0.727	0.722	0.916	0.705	0.846	0.916	0.003	-*	-	-	-	-	-	-	0.004	-	-	-	-	-
Heating	2.89	2.925	7.995	3.269	2.077	4.097	5.232	0.02	-	-	-	-	-	-	-	-	-	0.003	-	0.011	-
Microwave	0.381	1.143	3.768	10.31	0.261	1.062	2.635	0.01	-	-	-	-	-	-	-	-	-	-	-	0.094	-
Low pH	0.311	1.26	1.834	0.049	0.119	0.82	ND	-	-	-	-	0.01	-	-	-	-	-	-	-	ND	-
Sunlight	1.667	1.876	4.489	0.513	0.459	2.261	ND	-	-	-	-	0.004	-	-	-	-	-	-	-	ND	-
In-car-storage	1.635	0.696	1.313	0.372	0.48	0.895	ND	-	-	-	-	-	-	-	-	-	-	-	ND£	ND	ND

Treatment	PET							HDPE			PVC		LDPE			PP		PS	PC		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
Cooling	0.86	1.652	1.5	0.936	0.846	0.985	1.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heating	4.771	3.896	8.145	3.748	2.691	5.29	3.243	-	-	-	0.013	0.026	0.038	-	-	1.109	-	-	-	0.005	-
Microwave	0.391	1.502	4.748	10.51	0.511	1.904	3.694	0.004	-	-	0.015	0.005	-	-	0.001	0.007	-	-	-	0.1	-
Low pH	2.428	1.378	3.794	1.658	0.73	1.351	ND	0.007	-	-	-	0.01	-	-	0.005	0.001	-	-	-	ND	-
Sunlight	3.634	2.503	4.611	0.746	0.513	2.917	ND	-	-	-	-	0.013	-	-	-	0.02	-	-	-	ND	-
In-car-storage	2.475	1.328	3.08	0.771	0.52	1.212	ND	-	-	-	-	0.002	-	-	-	-	-	-	ND	ND	ND

-*: Data of samples was below detection limit
ND£: No sample was studied

It is reasonable to believe that microwaving may have similar effect as heating on metal leaching. From the results of Table 5, we can see that the antimony concentrations in bottles A to G treated with microwave increased from 0.381 to 10.51 μg/L relative to controls. The results are similar to those of bottles treated with boiling water. Bottle D showed the highest level of antimony leaching (10.51μg/L). For other metals in all bottle samples, no significant leaching was detected and it was far below the MCL of USEPA regulations, shown in Table 4. Similarly, the non-washed bottles have higher levels of antimony than those of washed ones for bottles A to G.

For the other bottles whose antimony was not used as catalyst, no obvious trends were found between with and without washing. Table 6 presented the differences of antimony leaching concentration between washing procedure involved and no washing

procedure involved (ΔC) in each treatment for all bottle samples. As clearly indicated in Table 6 , ΔC was above zero for every point which means more antimony leaching was detected in experiments with no washing procedure compared with the experiments with washing procedure. These interesting results indicate that some antimony was loosely attached to the plastic surface when they were newly manufactured and can be removed by washing. However, majority of antimony did leach from plastic upon treatment conditions change such as heating or microwave.

Table 6. Difference of antimony leaching between washing procedure involved and no washing involved for bottles A to G in experiments using cooling, heating, microwave treatment, low pH water, outdoor sunlight irradiation and in-car-storage treatments

	A	B	C	D	E	F	G
Low pH treatment	1.967	0.627	0.122	0.233	0.354	0.656	ND*
Outdoor sunlight irradiation	2.117	0.118	1.96	1.609	0.611	0.531	ND
In-car-storage	0.84	0.432	1.767	0.399	0.035	0.317	ND
Cooling treatment	0.185	0.925	0.778	0.019	0.141	0.139	0.124
Heating treatment	1.881	0.971	0.15	0.539	0.614	1.193	0.011
Microwave treatment	0.01	0.359	0.98	0.191	0.25	0.842	1.259

ND*: No sample was studied

Effects of low PH

It is always a big concern whether pH has any effect on metal contamination leaching from the plastic bottles. It has been reported that pH had no effect on antimony leaching over pH 6-8 which is typical ranges for drinking waters regardless of location

(Shotyk et al. 2006; Shotyk and Krachler 2007b; Shotyk and Krachler 2007a). However, it's still worth investigating whether metal leaching could happen at low pHs, because many fruit juices that are used in daily life, such as orange juice, apple juice, cranberry juice, may have very low pH values. Table 4 and Table 5 summarized the results testing bottles A to U filled with pH-adjusted water. In this study, pH=4.0 was used to simulate the pH of orange juice. The data showed that the antimony concentrations increased from 0.459 to 4.611 μg/L at low pH relative to controls in bottle samples A to G. Although they are below the MCL of USEPA regulation, it is still a concern for using PET bottles as orange juice bottles because antimony leaching does increase at acidic conditions. For other metals in all bottle samples, no significant leaching was detected and the levels of metals were far below the MCL of USEPA regulations. In addition, no correlation were found between results from experiments with washing procedure involved and without washing procedure for these 15 metal elements in samples A to G. However, as shown in Table 6, the ΔC is above zero for each bottle sample which means more antimony leaching was detected in experiment with no washing procedure compared with the experiment with washing procedure in bottles samples A to G.

Effect of outdoor sunlight exposure and in-car-storage

Outdoor sunlight irradiation has been studied for its effects on antimony leaching (Shotyk et al. 2006; Shotyk and Krachler 2007b; Shotyk and Krachler 2007a). However, its effect on other metal leaching was not studied. In this study, 16 potentially leaching metals including antimony upon outdoor sunlight irradiation were investigated. The results were shown in Table 4 and Table 5. The data reveal that, over the 7-days

exposures test, antimony concentration increased from 0.049 to 2.428 μg/L relative to control in bottle samples A to G which are below the MCLs of USEPA regulations. These results suggest that natural sunlight irradiation has only a minor effect on antimony leaching. For other 15 metals in all bottle samples, no significant leaching was detected or it's far below the MCL of USEPA regulations.

As we all know that temperature inside cars can exceed 45°C in the summer time in many US cities. Bottled water or other beverage storing in the car is very common practice. In this study, bottles were placed on the back seats in author's car for one week. Temperature inside the car was measured three times a day, the daily temperature ranged from 20 to 45 °C). The results were shown in Table 4 and Table 5. The results may have combined effects: sunlight irradiation and high temperature inside the car. The results indicated that, over the 7-day in-car-storage test, antimony concentration increased from 0.482 to 3.08 μg/L compared to the control in bottle samples A to G, even though the levels were below the MCL of USEPA regulations. These results suggest that high temperature storage enhances antimony leaching over a period of time. For other 15 metals in all bottle samples, no significant leaching was detected or it was far below the MCL of USEPA regulations.

Table 6 shows the antimony leaching concentration change between washing procedure involved and non-washing procedure involved for bottle samples A to G. Similar results were obtained. The ΔC is above zero for each sample which means more antimony leaching was detected in experiment with no washing procedure compared with that from experiment with washing procedure.

Conclusions

Several experiments were conducted to investigate factors that could potentially influence metal leaching from recycling plastic bottles, including cooling with ice cold water, heating with boiling water, Microwaving, incubation with low pH water, outdoor sunlight irradiation and in-car-storage. Total of 16 metals including antimony were examined in this study. The results revealed that heating and microwaving enhance antimony leaching significantly in PET plastic bottles (samples A to G); those manufactured using antimony as a catalyst. Incubation with low pH water, Outdoor sunlight irradiation and in-car-storage can also increase the antimony leaching significantly in this type of plastic, but to the lower scale than the boiling and microwaving. Cooling almost had no effect on antimony leaching based on our results. No significant leaching was detected or it was far below the MCLs of USEPA regulations for other 15 metals in all bottle samples studied. Another interesting result was that washing procedure did contribute to the antimony leaching in PET bottles. For bottles samples A to G which are made of PET, more antimony leaching was detected in experiments with no washing procedure compared with those with washing procedure which reveal that not all antimony leaching stem from condition changes but partially come from contaminations during production process. Therefore, plastic bottle manufacturers should consider the contaminations during manufacturing process and washing bottles before first use was strongly recommended to remove those contaminants.

Acknowledgments

We thank the Department of Chemistry and the Environmental Research Center at the Missouri University of Science and Technology for financial support

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3. Effect of oxidative and physical treatments on inactivation of *Cylindrospermpsis raciborskii* and removal of cylindrospermopsin

Abstract

The presence of toxic cyanobacterial blooms (or blue-green algae) in water bodies used either as drinking water or for recreational purposes may present serious health risks for the human population. In this study, the removal of the chemical toxin, cylindrospermopsin, via free chlorine, chlorine dioxide, monochloramine, permanganate, ozone, and UV irradiation was studied. Ozone and free chlorine were found to be highly effective for cylindrospermopsion removal while the other disinfectants were ineffective. Ozone and free chlorine were also determined to be highly effective for the inactivation of the cyanobacteria, Cylindrospermopsis raciborskii, at typical water treatment exposures, chlorine dioxide, monochloramine, and permanganate were only marginally effective at inactivation of Cylindrospermopsis raciborskii.

Keywords

cyanobacteria, cyanotoxin, cylindrospermopsin

Introduction

Concern about the effects of cyanobacteria (a blue-green alga) and their toxins in surface and drinking water on human and environmental health has grown throughout the world in recent years (Shaw et al. 1999; Fastner et al. 2003; Bouaicha & Nasri 2004; Nogueira et al. 2004; Fastner et al. 2007; Seifert et al. 2007; Yilmaz et al. 2008; Everson et al. 2009). It is estimated that 70% of these algal blooms are potentially toxic (Codd 1995; Ho et al. 2006). The occur- rence of cyanobacterial toxins can pose a risk for the health of both humans and animals. Cyanotoxins pose a technical challenge for water utilities when present in hazardous concentrations in water bodies used as a drinking water source (Newcombe & Nicholson 2004; Hoeger et al. 2004; Ho et al. 2008).

Cylindrospermopsin is a cytotoxic alkaloid produced by a range of cyanobacterial species in worldwide. Cylindrospermopsin was first identified in the species Cylindrospermopsis raciborskii from tropical waters, but is also produced by Aphanizomenon ovalisporum and Umezakia natans (van Apeldoorn et al. 2007). Cylindrospermopsin is zwitterionic, highly water-soluble, and stable to relative extremes of temperature and pH (Chiswell et al. 1999). The structure of cylindrospermopsin (molecular formula: C15H21N5O7S) is given in Figure 1. The

cylindrospermopsin analog, dexycylindrospermopsin, is commonly produced in varying proportions by the cyanobacteria that produce cylindrospermopsin. Cylindrospermopsis raciborskii is not only an invasive species, but is also a species with different physiological strains or ecotypes. Beginning 1997, Cylindrospermopsis raciborskii occurrence began to rapidly increase and to dominate some Florida (USA) water bodies (Carmichael et al. 2001). Its invasive behavior at mid-latitudes was also observed with Cylindrospermopsis raciborskii being reported in France, Germany, Hungary, Brazil, Austria, Greece, Slovakia, Portugal, Thailand, Mexico, and Senegal in the last decade (van Apeldoorn et al. 2007). In contrast of other cyanotoxins, a large proportion of cylindrospermopsin in environmental samples appears to be present in the extracellular form (van Apeldoorn et al. 2007). The level of cylindrospermopsin in environmental samples ranges from 0.1 to 20 mg/L in different water bodies (Duy et al. 2000; Saker & Griffiths 2001; Briand et al. 2002). The World Health Organization (WHO) has proposed a guideline of 1 mg/L for cylindrospermopsin.

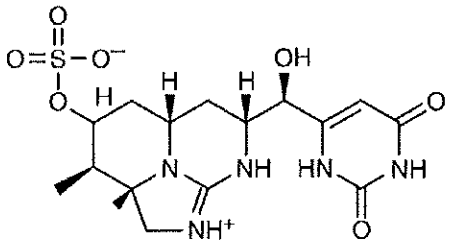

Figure 1. Molecular structure of cylindrospermopsin

Due to increasing occurrence and high toxicity, efficient treatment strategies are needed to prevent cyanotoxins occurrence in drinking water. Various oxidants and UV irradiation are commonly used for disinfection during drinking water treatment. In the disinfection process, the intracellular toxin could be released into water resulting in increased concentrations in the aqueous phase. Ultimately, the goal of disinfection of cyanobacteria and cyanotoxins in drinking water is to inactivate the bacteria, and degrade both the intra- and extra-cellular cyanotoxin.

In this study, the removal of the toxin, cylindrospermopsin was studied from aqueous solution for each of six disinfectants at varied exposures to examine the effectiveness for treating cylindrospermopsin if present in source water for a water treatment plant. Additionally, the inactivation of the cyanobacteria, Cylindrospermopsis raciborskii, was studied with each of the six disinfectants.

The purpose of this study was also to examine the potential for release of cylindrospermopsin into the water during cyanobacteria inactivation through cell leasing or increased permeability of the intracellular cylindrospermopsin.

Experimental

General reagents

All chemicals and reagents used in this study were analytical grade or better unless otherwise stated. Cylindrospermopsin standards were purchased from Alexis Biochemicals Corporation (San Diego, C A , USA). Cylindrospermopsin stock

solutions were prepared in methanol. Saline (0.9% NaCl) and NaCl solutions of other concentrations were prepared by dissolving NaCl in deionized water which was produced with a Millipore Simplicity 185 water system (Billerica, MA). A buffered saline stock solution was made by dissolving 0.12% NaH_2PO4 and 0.78% NaCl in deionized water with pH adjusted to 7.6.

Oxidants solution preparation

The free chlorine stock solution was prepared by dilution from 5% sodium hypochlorite solution (Fisher Scientific). The free chlorine concentration was determined using HACH DPD Method 8021. The monochloramine stock solution was prepared from mixing sodium hypochlorite and ammonium chloride at a molar ratio of 1:1.05 at greater than pH 9. Monochloramine concentration was determined using HACH Nitrogen, Free Ammonia, and Chloramine (Mono) Indophenol Method 10200. The permanganate stock solution was prepared by dissolving potassium permanganate in deionized water.

Permanganate concentration was determined using HACH DPD Total Chlorine Method 8167. Gaseous chlorine dioxide was generated by a CDG Bench Scale ClO2 Generator (CDG, Bethlehem, PA). The gaseous chlorine dioxide was bubbled through a stone diffuser into a receiving solution of pH pre-adjusted phosphate buffered deionized water to produce a homogenous chlorine dioxide stock solution. The chlorine dioxide concentration was determined using HACH DPD Method 8167.

Gaseous ozone was generated by an ozone generator (Model GLS-1, PCI-WEDECO Environmental Technologies, West Caldwell, NJ). The gaseous ozone was bubbled through a stone diffuser into a receiving solution of pH preadjusted phosphate

buffered deionized water to produce a homogeneous ozone stock solution. The ozone concentration was measured using a conventional spectrophotometer (Cary 50 Conc., Varian) at 260 nm.

Culturing of *Cylindrospermopsis raciborskii*

Cylindrospermopsis raciborskii (Strain UTEX LB2897) and DYIII medium were purchased from the culture collection of algae at University of Texas at Austin (Austin TX, USA). The cells were cultured in flasks placed on a shaker table at a speed of 30 rpm under periodic simulated sunlight. Specifically, cool-white fluorescence lamps were used to provide 30 mE·m22·s21 solar spectral light. The light/dark cycle was set at 12 hr/12 hr each day. The cell cultures were split every six weeks with 20mL old cell solutions transferred to 180mL DY-III culture media. The temperature was controlled to 22(±1)°C.

Cell viability

The cells growing in the culture media were counted regularly to monitor their growth. Before each treatment experiment, the cell concentration was measured by direct counting with a hemacytometer (Fisher Scientific) using a Leica Gallen III compound microscope. The cell viability was determined using a Simplate for HPC (IDEXX Laboratories, Inc.) immediately before and after each treatment. Briefly, treated cells were diluted and mixed with the medium (WHPC-100) at a ratio of 1:9 in each Simplate. In the method, viable (live) cells fluoresce under a UV light (6 watt, 365 nm) after 48 hours of incubation at 358C, while non-viable (inactivated or dead) cells do not.

The viability of each treated cell sample was obtained by determining the Most Probable Number (MPN) of cyanobacteria in the original sample referring to the MPN table provided by Simplatew for HPC.

LC-MS/MS analysis of cylindrospermopsin

Analysis of cylindrospermopsin was performed using triplequadrupole mass spectrometer using an Applied Biosystems 4000QTRAP equipped with an Agilent 1100 series LC system. The analytical column used for chromatographic separation was a Phenomenex Synergi 3.0 £ 150mm 4mm.The separation was achieved with a 95% Eluent A (deionized water with 0.1% (v/v) formic acid) and 5% Eluent B (acetonitrile with 0.1% (v/v) formic acid). The total flow rate was 0.2 mL/min and an injection volume of 15ml was used. Quantification of cylindrospermopsin was achieved using the 413.81/272.1 transition ion pair. A typical ion chromatogram for cylindrospermopsin is shown in Figure 2. The detection limit using this method was typically 0.05 mg/L, with a linear response up to 100 mg/L.

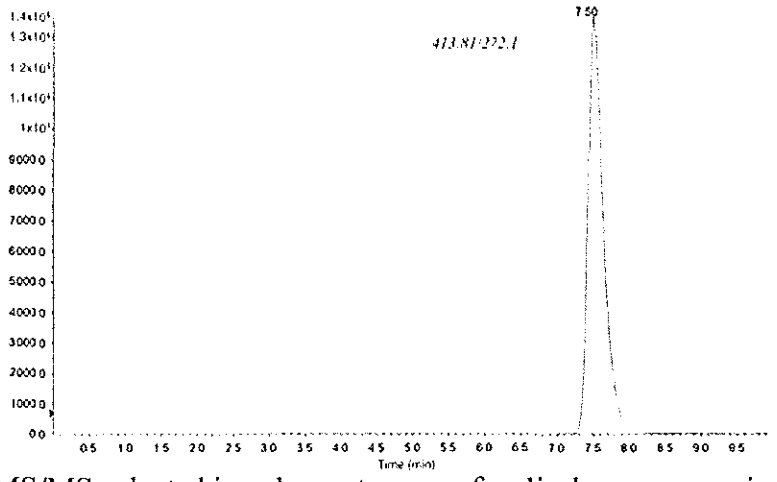

Figure 2. LC-MS/MS selected ion chromatogram of cylindrospermopsin in reagent water

Oxidative and physical treatments

Bacterial cells for each treatment were always treated identically and taken at same stage. Specifically, 4–6mL of the cell suspensionwas always taken from culture flask three weeks after subculturing for use in an experiment. After centrifugation at 1,500 rpm in a Clay Adams SEROFUGE II centrifuge for 3 minutes, the supernatant was removed and the cell pellet was re-suspended in 1mL of saline solution. Culture medium, dissolved matter and any floating cells were removed after repeating the above procedure three times. After the final wash, the cell pellet was resuspended in saline solution. Next, 2mL of the cell suspension (density of 2×10^5 cells/mL) was used for each treatment experiment. For each oxidation experiment, the oxidant stock solution was spiked into prepared cell suspension to 2 mg/L oxidant, followed by immediate tumbling for specific reaction periods. The reaction vials were wrapped with aluminum foil to prevent any light-induced degradation of an oxidant during treatment. After a specific reaction time, a 10% overdose of ascorbic acid stock solution was spiked into the reaction vial to quench the oxidant. In the UV treatment experiments, UV irradiation was produced using a Pen Ray 1-W low-pressure narrow-band mercury vapor lamp (Model 90-0004-01) (254 nm). The UV lamp was totally submerged in the cell suspension.

Results and Discussions

Intra- versus extracellular cylindrospermopsin

The extra- and intra-cellar fraction of cylindrospermopsin was determined in the Cylindrospermopsis raciborskii cultures used in the experiments, specifically in cell

pellet, medium and floating cells. Briefly, 2mL of the cell suspension was taken directly from the culture at the same stage as other treatment experiments, and counted. After centrifugation, the cylindrospermopsin was partitioned into three fractions: cell pellet, medium, and floating cells. The medium was centrifuged again using a 0.22-mm filter, and analyzed using LC-MS/MS without dilution. The cell pellet was resolved in 6mL of methanol, while the floating cells were resolved in 2mL of methanol. After one hour to assure the cells were completely broken by methanol, the samples were centrifuged using a 0.22μm filter, followed by LC-MS/MS analysis.

In the cultures used in this work, the intracellular cylindrospermopsin concentration was determined to be $0.034(10^{-6})$ mg/cell Previous work showed that intracellular cylindrospermopsin content may vary from a minimum of $0.002(10^{-6})$ to a maximum of $0.055(10^{-6})$ mg/cell at different stages of culturing (Chiswell et al. 1999).

Free chlorine oxidation

Free chlorine (HOCl/OCl2) was highly effective at the oxidation of cylindrospermopsin from homogeneous solution (Figure 3A). Specifically, the experiments with free chlorine (HOCl/OCl2) at pH ¼ 8 at exposures of 0, 4, 10, 60, 120, 240, and 360mg·min/L (and 1 mg/L concentration) showed that the half life of cylindrospermopsin was less than 1.7 min with a free chlorine concentrations of 1 mg/L. The experiments with free chlorine also showed that 100% inactivation of Cylindrospermopsis raciborskii was achieved with exposures as low as 4mg·min/L (Figure 3B). No cylindrospermopsin was observed in solution as a result of free chlorine oxidation (Figure 3B). This could be due either to the lack of release of

cylindrospermopsin due to oxidation, or to the rapid oxidation of any cylindrospermopsin that was released. For comparison, typical drinking water treatment exposures for 3-log inactivation of Giardia cysts are in the range of 45–75mg·min/L (2003b).

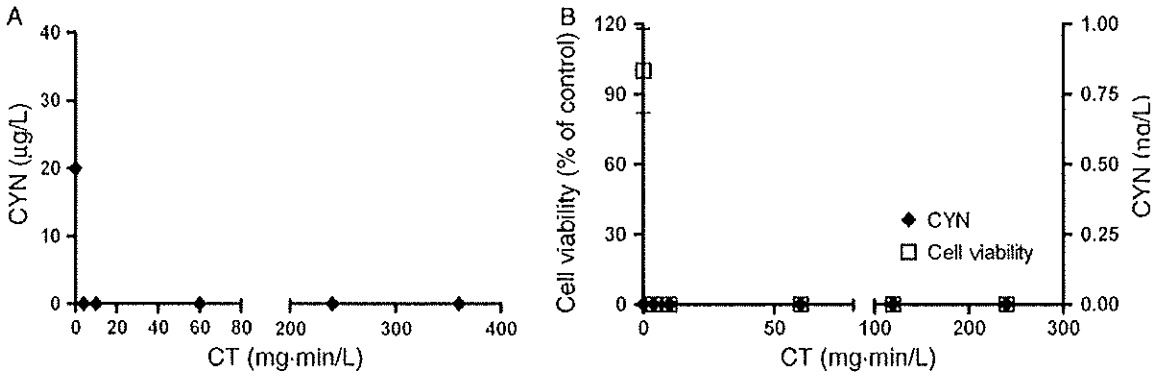

Figure 3. (A) Free chlorine oxidation on aqueous cylindrospermopsin solution, (B) Free chlorine oxidation on *Cylindrospermopsis raciborskii* showing both cell viability and cylindrospermopsin concentration.

Free chlorine at typical drinking water treatment exposures appears to be a highly effective means at both oxidation of cylindrospermopsin, as well as disinfection of Cylindrospermopsis raciborskii.

Chlorine dioxide oxidation

For chlorine dioxide (ClO2), cylindrospermopsin was recalcitrant towards oxidation from homogeneous solution (Figure 4A). Specifically, chlorine dioxide exposures as high as 480mg·min/L resulted in no removal of cylindrospermopsin. This is consistent with results by Rodriguez (Rodriguez et al. 2007a,b) who showed cylindrospermopsin oxidation by chlorine was a slow processes with a half life of 14.4 hr

with 1 mg/l chlorine dioxide at pH =8.0. For comparison, typical drinking water treatment exposures for 3-log inactivation of Cryptosporidium and Giardia cysts at 20–25°C are 11–15 and 226–347mg·min/L, respectively (2003a).

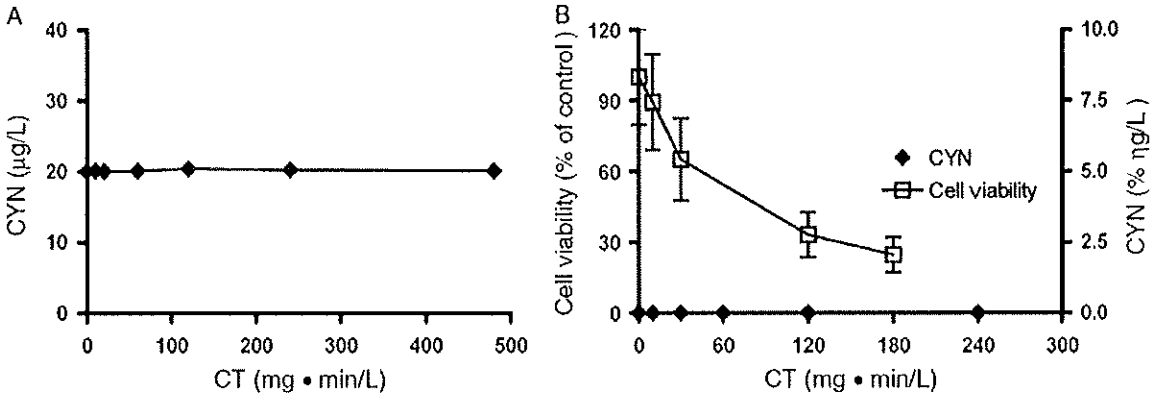

Figure 4.(A) Chlorine dioxide oxidation on aqueous cylindrospermopsin solution, (B) Chlorine dioxide oxidation on *Cylindrospermopsis raciborskii* showing both cell viability and cylindrospermopsin concentration.

The results also showed that chlorine dioxide exposure on the order of 180 mg·min/L, greater nearly 75% inactivation of Cylindrospermopsis raciborskii was achieved. No concurrent buildup of cylindrospermopsin was observed in solution. Because cylindrospermopsin was shown to not be oxidized from solution with chlorine dioxide, these results suggest that the toxin is not released from the cyanobacterial cell during oxidative inactivation with chlorine dioxide. Chlorine dioxide at typical drinking water treatment exposures does not appear to be an effective means of control of cylindrospermopsin nor of disinfection of Cylindrospermopsis raciborskii.

Monochloramine oxidation

For monochloramine (ClNH2) oxidation, the cylindrospermopsin was highly recalcitrant showing nearly no removal with exposures of up to 360mg·min/L (Figure 5A). For comparison purposes, typical drinking water treatment exposures for monochloramine for 3-log inactivation of viruses and Giardia cysts at 20–258C is about 500–750 and 750–1,100mg·min/L, respectively (2003a). With monochloramine exposures on the order of 240 mg·min/L partial inactivation of *Cylindrospermopsis raciborskii* was observed (Figure 5B). No concurrent accumulation of cylindrospermopsin was observed in solution indicated it was not released from the bacterial cell during monochloramine oxidation. Monochloramine at typical drinking water treatment exposures does not appear to be an effective means of control of cylindrospermopsin, and only partially effective for the inactivation of *Cylindrospermopsis raciborskii*.

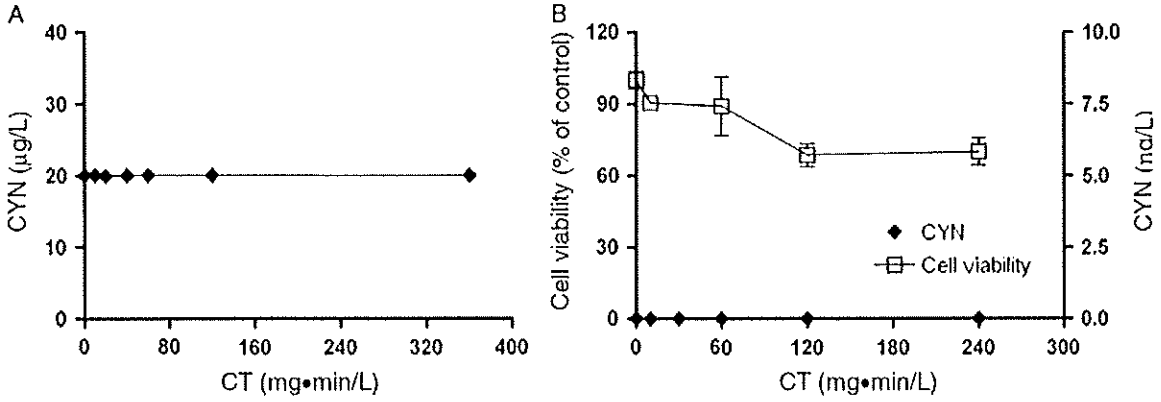

Figure 5.(A) Monochloramine oxidation on aqueous cylindrospermopsin solution, (B) Monochloramine oxidation on *Cylindrospermopsis raciborskii* showing both cell viability and cylindrospermopsin concentration.

Permanganate oxidation

Similar to monochloramine, permanganate was ineffective at the oxidation of cylindrospermopsin at exposures up to 360mg·min/L (Figure 6A). These results are consistent with Rodriguez (Rodriguez et al. 2007a,b) who determined a half life for cylindrospermopsin of greater than 4 days at 1 mg/L permanganate. For comparison, typical permanganate exposures in water treatment may be on the order of 100–200mg·min/L, although higher exposures as certainly possible such as when there are long contact times between an inlet to a treatment plant and the plant itself. Permanganate oxidation was observed to achieve partial inactivation of *Cylindrospermopsis raciborskii* with exposures of up to 240mg·min/L (Figure 6B). No concurrent buildup of cylindrospermopsin was observed in solution which suggests that it was not released from the cyanobacterial cell during inactivation. Thus, permanganate at typical drinking water treatment exposures does not appear to be an effective means of control of cylindrospermopsin, and only partially effective for the inactivation of *Cylindrospermopsis raciborskii*.

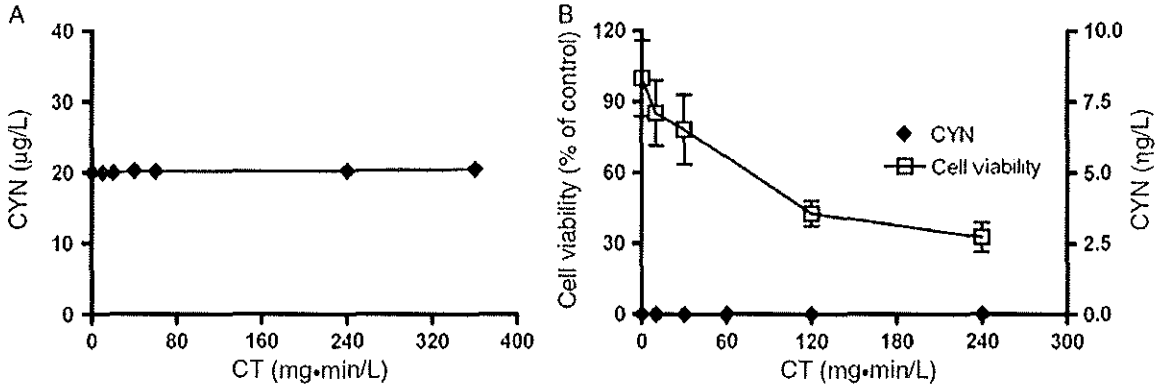

Figure 6.(A) Permanganate oxidation on aqueous cylindrospermopsin solution, (B) Permanganate oxidation on *Cylindrospermopsis raciborskii* showing both cell viability and cylindrospermopsin concentration.

Ozone oxidation

Ozone was observed to be highly reactive with cylindrospermopsin with exposures as low as 1mg·min/L causing the complete removal of cylindrospermopsin (Figure 7A). This result is consistent with results by Rodriguez (Rodriguez et al. 2007a,b) who found a half life of just 0.1 s at 1 mg/L of ozone at pH ¼ 8. For comparison, 3-log inactivation of Cryptosporidium and Giardia cysts at 20–258C is achieved with 7.4–12 and 0.5–0.7mg·min/L, respectively (2003a). Similarly, exposures of just 1mg·min/L achieved complete inactivation of *Cylindrospermopsis raciborskii* with no buildup of cylindrospermopsin in solution (Figure 7B). This is due either to the lack of release of cylindrospermopsin during the oxidation process or to the near instant oxidation of the cylindrospermopsin after release. Ozone at typical drinking water treatment exposures appears to be a highly effective means at both oxidation of cylindrospermopsin, as well as disinfection of *Cylindrospermopsis raciborskii.*

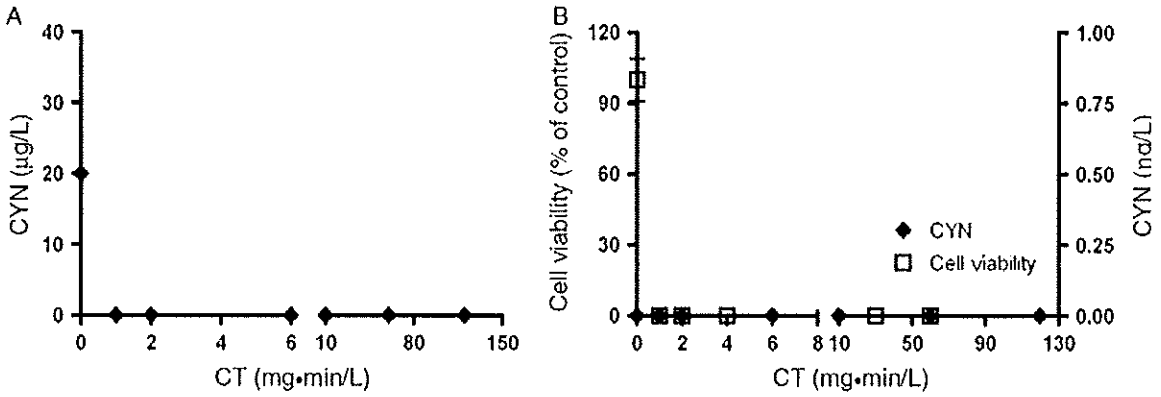

Figure 7. (A) Ozone oxidation on aqueous cylindrospermopsin solution, (B) Ozone oxidation on *Cylindrospermopsis raciborskii* showing both cell viability and cylindrospermopsin concentration.

UV irradiation

Treatment with UV has been widely used for destruction and removal of organic compounds from water supplies. The UV disinfection on cylindrospermopsin was suggested to occur via a mechanism involving dissolved organic radicals and most efficient at higher pH levels (Griffiths & Saker 2003). In our study, UV doses of 0, 128, 257, 643, 1,287 and 3,861 mJ/cm2 were applied in duplicate experiments. Figure 8A shows degradation of cylindrospermopsin does occur, but at UV doses many times that used in water treatment disinfection. Specifically, common UV dosages used for 3-log removal of Giardia and Cryptosporidium are just 11 and 12 mJ/cm2, respectively (2003b). Similarly, UV irradiation was observed to achieve *Cylindrospermopsis raciborskii* inactivation, but not at dosages common to water treatment (Figure 8B). Thus, UV irradiation at typical drinking water treatment exposures does not appear to be an effective means to remove cylindrospermopsin nor to inactivate *Cylindrospermopsis raciborskii.*

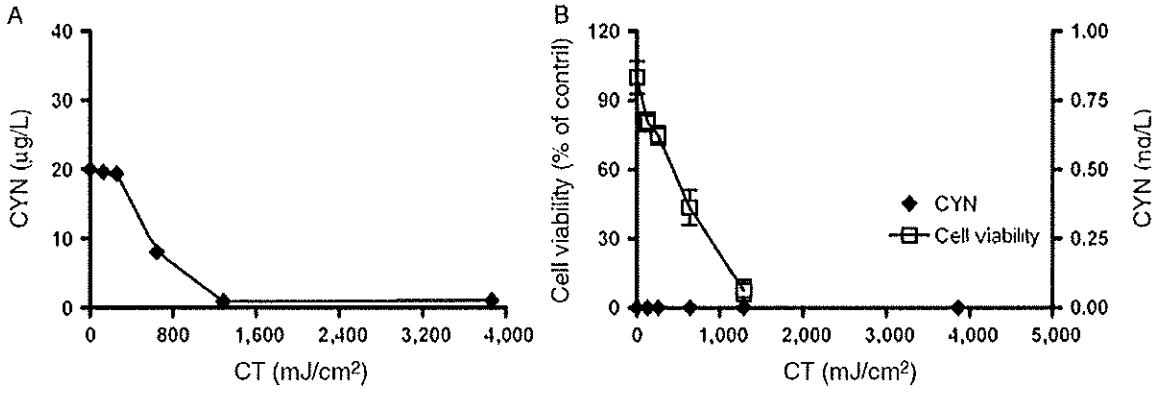

Figure 8.(A) UV irradiation treatment on aqueous cylindrospermopsin solution, (B) UV irradiation treatment on *Cylindrospermopsis raciborskii* showing both cell viability and cylindrospermopsin concentration.

Conclusions

In this study, several oxidative and UV irradiation disinfection treatments were examined to study the removal cylindrospermopsin from homogeneous solutions, the inactivation efficiency each disinfectant for *Cylindrospermopsis raciborskii.,* and the potential for release and buildup of cylindrospermopsin in the aqueous solution. The results showed the ozone and free chlorine were highly effective at the control both of cylindrospermopsin and of *Cylindrospermopsis raciborskii.* Chlorine dioxide, monochloramine, permanganate, and UV irradiation at typical water treatment dosages were all ineffective at removing the chemical toxin, cylindrospermopsin. Chlorine dioxide, monochloramine, and permanganate were each only capable of partial inactivation of *Cylindrospermopsis raciborskii.* In no case did the disinfection or oxidation of *Cylindrospermopsis raciborskii* cause the buildup of cylindrospermopsin in solution. This information provides the basis for control of both *Cylindrospermopsis raciborskii* and cylindrospermopsin in water treatment plants.

Acknowledgments

We thank the Department of Chemistry and the Environmental Research Center at the Missouri University of Science and Technology, and Missouri Department of Natural Resource for financial support. Sincere thanks are extended towards Chuan Wang and Jie Ding for their technical assistance during experimental and analytical portions of this work.

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4. LC-MS/MS determination of cyanobacterial toxins in water samples

Abstract

The presence of cyanobacterial and their toxins in water bodies have emerged as a worldwide concern due to the perceived increase in occurrence and severity. There is a need for simple, inexpensive methods for rapid screening of cyanotoxins in a wide variety of water types. This work developed a fast and easy method for quantitative analysis of nine major cyanotoxins using liquid chromatography-tandem mass spectrometry without sample cleanup processes such as solid phase extraction. The method limit of quantification ranges of 0.002-0.1 μg/L and a good linearity was obtained over a concentration range of 0.02-100 μg/L. The method has been successfully applied to different water matrix including reagent water, river water and wastewater in a reproducible manner. The quantitative analyses show a precision with RSDs of around 6% to 17% in untreated river water and 9.9% to 18.3% in treated wastewater. It also screened 68 water samples, both untreated source water and treated, from 34 different water treatment plants cross Missouri for cyanotoxins. Samples were collected from several water resources, including the Missouri river, the Mississippi River, groundwater, lakes, reservoirs, and wells. However cyanotoxins were detected below limit of quantification in all samples.

Keywords

Cyanotoxins, mass spectrometry, occurrence

Introduction

The presence of cyanobacteria in water resources has received considerable attentions in the past two decades. Cyanobacteria periodically bloom in water bodies in nationwide, a variety of cyanobacteria and their toxins were identified and their occurrences were reported in fresh, brackish and marine waters all over the world[1-9]. A survey reported that 70% of these algal blooms are potentially toxic by releasing cyanotoxins[10, 11]. The presence of cyanotoxins in surface or drinking water may cause serious health risks to humans and animals.

The major cyanotoxins include cylindrospermopsin(CYN), microcysitins(MCs) and saxitoxins(STXs)[12]. MCs are the most common cyanotoxins which can be produced by several cyanobacteria such as *Microcystis, Anabaena and Nostoc.* Microcysitins have been found in many countries including Australia, Canada, China, Holland, and US, and the toxin levels were reported from 0.3 to 80 μg/L. Of all the MCs, MC-LR is the most abundant and the most toxic making up 45.5% to 99.8% of total MCs concentration in natural water[10-12]. CYN was firstly identified in the species *Cylindrospermopsis raciborskii* which have began to rapidly increase and dominate some Florida water bodies since 1997. This chemical is highly water soluble and stable to relative extremes of temperature and pH[12-17]. STXs are representative of a large toxin family referred to as the

paralytix shellfish poisoning toxins. These toxins are identical to those produced by some toxgenic marine dinoflagellates that accumulate in shellfish that feed on those algae, It's the most powerful marine toxin currently know and among the most dangerous poisons on Earth. STX and neo-STX have been reported in freshwater cyanobacteria including *Aphanizomenon spp.* and *Lyngbya wollei*[12, 18, 19].

The United States Environmental Protection Agency (EPA) has included "cyanobacteria (blue– green algae), other freshwater algae, and their toxins" in its Contaminant Candidate List as one of the microbial drinking water contaminants targeted for additional study, but it does not specify which toxins should be targeted for study[20].Based on toxicological, epidemiology and occurrence studies, the EPA Office of Ground Water and Drinking Water has restricted its efforts to 3 of the over 80 variants of cyanotoxins reported, recommending that Microcystin congeners LR, YR, RR and LA, and Cylindrospermopsin be placed on the Unregulated Contaminant Monitoring Rule (UCMR)[21]. The world Health Organization recently proposed a provisional upper limit in drinking water of 1 μg/L for MC-LR.

More effective protection to water resources requires efficient detection of the whole spectrum of cyanotoxins, there is an immediate need for rapid techniques for both screening and confirmatory methods for the cyanotoxins analyses. Instrumental methods are needed for use where quantitation and specificity are important. The most sensitive technique currently used for the analysis of trace-level concentrations in water samples involves liquid chromatography-mass spectrometry, specifically LC/MS/MS, which has been widely applied in environmental analysis[22, 23]. A range of LC/MS/MS methods for cyanotoxins have been developed[24-27], but none have been accepted a validated US EPA

methods or consensus organization methods. Most of these methods are dependent on sample cleanup methods such as solid phase extraction which require high solvent volumes and usually have low recovery rates. There is a need for simple, inexpensive methods for rapid screening of cyanotoxins in a wide variety of water types.

The objective of present study was to develop a fast, accurate, and easy method for quantitative analysis of nine major cyanotoxins using LC/MS/MS, and validated this method in various water matrixes including reagent water, untreated river water and treated wastewater. This research also screened 68 water samples, both untreated source water and treated water, from 34 water treatment plants in Missouri.

Experimental

General reagents

All chemicals and reagents used in this study were analytical grade or better unless otherwise stated. Cylindrospermopsin, Microcystin-LA, Microcystin-LF, Microcystin-RR, Microcystin-YR, Microcystin-LR standards were purchased from Alexis Biochemicals Corporation (San Diego, CA, USA). The Saxitoxin, dc-Saxitoxin, neo- Saxitoxin standards were purchased from the Institute for Marine Biosciences (National Research Council of Canada, Ottawa, Ontario, Canada).

Standard solutions and quality-control samples

Nine studying cyanotoxins were shown in Table 1. Stock solutions were prepared with methanol, and solutions of other concentrations were prepared by diluting with

Milli-Q water produced with a Millipore Elix-3 system (Billerica, Massachusetts). Stock solutions of standards were prepared at a concentration of 10 μg/mL and working solutions were made up at concentrations in the range from 0.1 to 500μg/L. All solutions of standards were stored at -20 °C until required and all were stable for a minimum of 3 months. Samples used for calibration and quality-control purposes were prepared freshly prior to analysis.

Table 1. Studying cyanotoxins

Compound	Abbreviation	MW
Cylindrospermopsin	CYN	415
Saxitoxin	STX	299
neo-Saxitoxin	neo-STX	315
dc-Saxitoxin	dc-STX	256
Microcystin-LR	MC-LR	994
Microcystin-RR	MC-RR	1037
Microcystin-YR	MC-YR	1044
Microcystin-LA	MC-LA	909
Microcystin-LF	MC-LF	983

Sample collection, storage and sampling location

Water samples were collected in precleaned amber glass bottles. For river water collection, a large precleaned wide mouth bottle or beaker was used to collect water at a representative area. For tap water collection, the water was allowed to flow for about 5 min. Sample bottles were filled from the container to just overflow, Sealed and placed in a cooler with ice for overnight shipment to the lab. The samples were filtered through a 0.45 μm nylon membrane filter and stored in refrigerator until analysis at 4°C. The analysis was completed within a week after collection. Water samples were collected

across the Missouri state. A total of 68 water samples were collected from a variety of water resources, including Missouri river, Mississippi River, and various lake water, reservoir water, and underground wells. Both untreated source and treated source water samples from each water treatment plant were analyzed.

Instrumentation

The HPLC system consisted of an Agilent 1100 pump/autosampler and a reversed phase C-18 column. The mass spectrometer was an API 4000 Q Trap equipped with an electrospray ionization interface. An automated switching valve was used between the HPLC and mass spectrometer (MS) to direct the mobile phase to the waste or MS. Amber glass sampler vials were used for all samples and the tubing used is PEEK material. The software program that provided the data platform for spectral acquisition and peak quantification was analyst 1.4.

HPLC system

The chromatographic separation was performed on a Phenomenex Synergi C-18 (3.0mm×150 mm i.d, 4μm particles) analytical column at a flow rate of 0.3 mL/min with an analysis time of 16mins, and the injection volume was 20 μl. Both the autosampler and column were kept at room tempreture (~25 °C). Separation was achieved by a gradient elution program with solvent A(Milli-Q water with 0.1 formic acid and 2.5mm ammonium formate) and solvent B(Methanol with 0.1 formic acid and 2.5mm ammonium formate): started with 10% B; increased to 70% B over 1min and to 78%B

over 1min; increased to 100% B over 9mins and decreased to 10% B over 0.1min and equilibrated at 10% B for 5 min, prior to the next injection, the total run time was 16min.

MS system

Tandem mass spectra were acquired on a triple quadrupole instrument. Positive electrospray ionization combined with the multiple reaction monitoring (MRM) mode was used. The curtain and collision gas flows were set to 25 l h^{-1} and medium level, the ion spray voltage was operated at 3000V with a source temperature of 450°C. A dwell time of 120ms was used per ion pair monitored. Nitrogen for the curtain and collision gas was generated by a *Peak* Scientific N_2 Generator. Table 2 summarizes the instrumental conditions and method parameters.

Table 2. MS parameters for determination of cyanotoxins in MRM mode

MS parameter	CYN	STX	neo-STX	dc-STX	MC-LR	MC-RR	MC-YR	MC-LA	MC-LF
Ion transitions	416/194	300/204	316/55	257/126	498/227	520/135	523/135	910/135	493/289
Collision gas(l h^{-1})	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Polarity	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Curtain gas(l h^{-1})	25	25	25	25	25	25	25	25	25
Dwell time(ms)	110	110	110	110	110	110	110	110	110
Ion spay voltage(V)	5000	5000	5000	5000	5000	5000	5000	5000	5000
Heater temperature(°C)	450	450	450	450	450	450	450	450	450
Declustering potential(V)	71	76	76	76	51	51	61	126	56
Collision cell exit potential(V)	12	12	8	6	14	8	8	6	20
Entrance potential(V)	10	10	10	10	10	10	10	10	10
Collision energy(V)	51	35	101	29	27	41	19	85	25

Results and Discussions

LC-MS/MS method optimization

In the initial stages of developing a method of detection for cyanotoxins, several solvents were tested in order to determine the optimal mobile phase. Cyanotoxins dissolve readily in solution containing ammonium formate but do not fully dissolve in pure acetonitrile. Thus ammonium formate was incorporated into the HPLC mobile phase. Methanol was selected as the organic constituent of the mobile phase with formic acid added to improve the signal strength. A mobile phase consisting 2.5mm ammonium formate and 0.1% formic acid resulted in optimal retention time and peak shape. A total nine cyanotoxins were separated and detected within 17min using this method. A representative MRM LC/MS/MS chromatogram of cyanotoxins in reagent water is shown in Figure 1. The first compound eluted at ~2.2min, and last at 10.5min. Because neo-STX and ds-STX have very similar chemical structures, it's hard to separate them at high resolution meanwhile keeping the method working for other analytes, same thing happened on M-LF and M-LR. However, the co-eluting compounds can be differentiated by different MRM transitions. Other cyanotoxins were well separated chromatographically and peak showed very good symmetry.

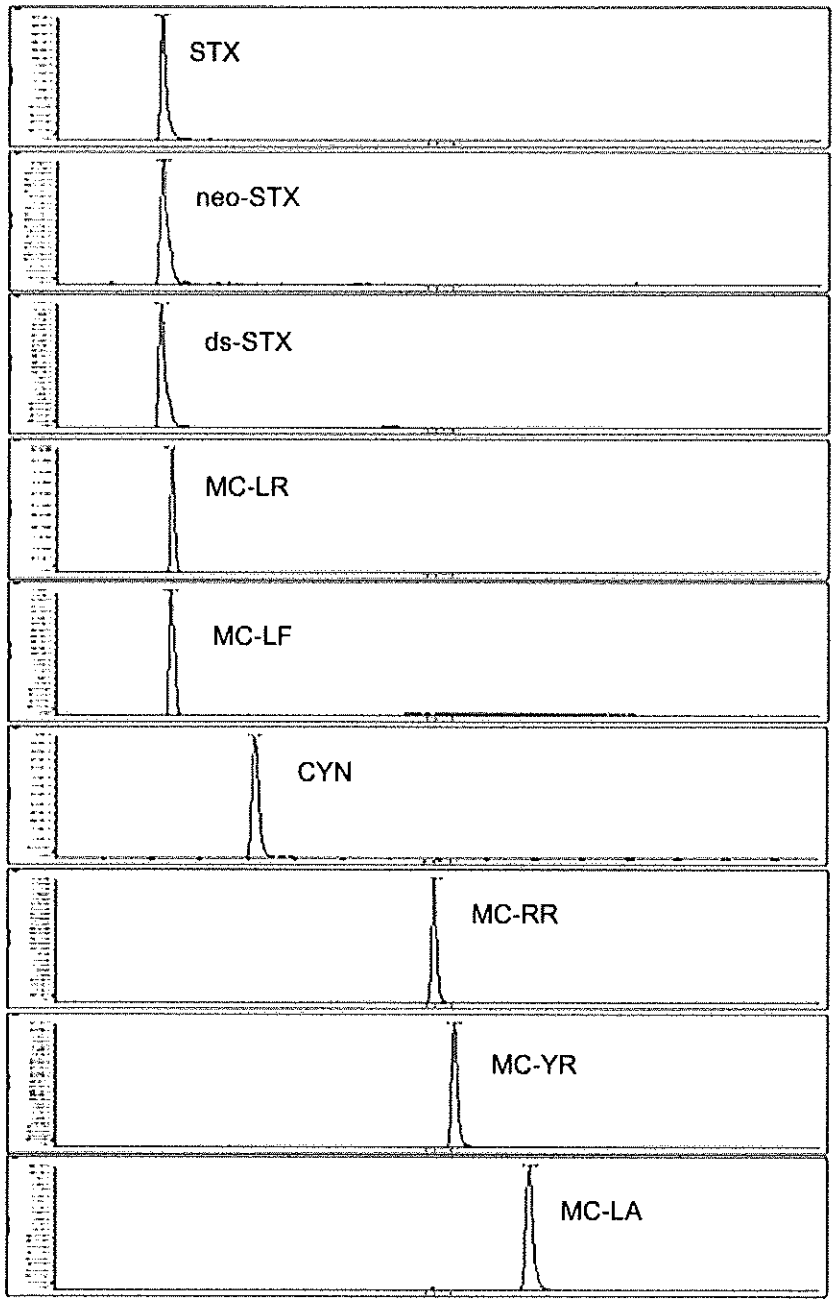

Figure 1. MRM LC/MS/MS chromatogram of cyanotoxins in reagent water

The mass spectrometry was operated in multiple reaction monitoring mode at an optimized voltage for each transition in positive mode. To select the MS/MS parameters,

standard of cyanotoxins were injected in continuous-flow mode and the declustering potential, collision energy, and collision cell exit potential were optimized for each transition. The curtain and collision gas flows were 25, 40 for gas1 and 60 for gas2. And the ion spray voltage was operated at 5000V with a source temperature of 450°C. A dwell time of 110ms was used per ion pair monitored. The nitrogen gas was generated by Peak scientific N_2 generator. For all analytes, the precursor ion detected was the $[M+H]^+$ or $[M+2H]^{2+}$ ion. The most abundant transition was used for quantitation. In ESI mode, CYN and STX form mainly $[M+H]^+$ ion which further loss of H_2O and NH_3, and for detection of MCs, $[M+H]^+$ was applied on M-LA, $[M+2H]^{2+}$ was dominant transitions for other MCs in this study. The guanidine group in the arginine residue is the preferred protonation site in MCs and it determines the ionization state. In case two arginine residues are present, doubly charged ions are formed. The ion transition for each analyte was present in Table 2

LC-MS/MS method detection limit and quantification

The limit of detection (LOD) for each analyte was determined following the USEPA standard method in preliminary test. Specifically, seven spike replicates were analyzed at a concentration of 2-5 times the estimated instrument detection limit, with LOD calculated as the product of the standard deviation(s) and student's t (α=0.01, d.f=6). However, because the instrument is sensitive and stable, this calculated LOD was too low to achieve. Thereafter, LOD for each cyanotoxin was determined as the lowest injected standard that gave a signal-to-noise (S/N) ratio between at 3. The S/N ratio was calculated by measuring the peak height to averaged background noise ratio. The

background noise was based on the peak-to-peak baseline near the analyte peak. The method LODs for this group of cyanotoxins were between 0.002 and 0.1 μg/L in reagent water. Similarly, limit of quantification (LOQ) for each studying cyanotoxin was obtained as the lowest injected standard that gave S/N ratio at 10, the method LOQs range between 0.02 and 0.5μg/L. A six-point standard calibration standards, at concentration ranges of 0.02-100 μg/L, were analyzed using linear regression with inverse weighting (1/x). Satisfactory r^2 values were obtained for analytes. Blanks processed through the entire method were also analyzed with each sample set. The validation results of overall method were listed in Table 3.

Table 3. The validation results of overall method

Cyanotoxins	LOD (μg/L)	LOQ (μg/L)	Linearity		2hrs at 25°C		7days at -20°C	
			Range (μg/L)	r^2	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
CYN	0.1	0.5	0.5~100	0.987	6.7	101	9.2	98.4
STX	0.05	0.5	0.5~100	0.999	8.4	104	6.3	95.3
Neo-STX	0.1	0.5	0.5~100	0.992	8.2	110	8.3	90.5
Dc-STX	0.05	0.5	0.5~100	0.998	3.5	95.3	7.2	91.6
M-LA	0.1	0.5	0.5~100	0.995	9.3	99.1	3.4	93.2
M-LF	0.002	0.02	0.02~100	0.995	2.4	103	4.6	89.8
M-RR	0.1	0.5	0.5~100	0.998	6.5	105	8.1	88.4
M-YR	0.1	0.5	0.5~100	0.997	5.5	108	7.9	92.5
M-LR	0.002	0.02	0.02~100	0.991	4.9	102	6.4	94.6

Accuracy, precision and stability

The precision of the method was evaluated by determining the relative standard deviation (RSD) of spiked samples. The RSD were obtained from multiple (n=3) analyses, For analyte-free reagent water spiked with 5μg/L cyanotoxins standards, respectively, RSD ranged from 1.46% to 8.32%, with a median of 3.99%.

To test the method accuracy, recoveries for analyte spikes were conducted. The recoveries were obtained from multiple (n=3) analyses, For analyte-free reagent water spiked with 5 μg/L cyanotoxins standards, spiking recoveries ranged from 91.9% to 110%. These recoveries are well within the commonly accepted range of 70-130% indicated in the USEPA method .The RSDs and recovery results were listed in Table 4.

The stability data are shown in Table 3, cyanotoxins was determined to be stable under different temperature and storage conditions. 20 μg/L cyanotoxins standards in reagent water were subjected to short term room temperature conditions for 2h, long term storage conditions for 7 days(-20°C). All samples evaluated displayed variability of less than 10% RSD.

Table 4. Accuracy and precision results in various water matrices

Cyanotoxins	5 μg/L in reagent water		5 μg/L in river water		5 μg/L in wastewater	
	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
CYN	2.7	110	14.5	135	9.9	123
STX	3.5	104	8.97	129	15.3	115
Neo-STX	6.2	98.5	13.6	132	17.9	132
Dc-STX	2.3	105	6.21	115	11.9	119
M-LA	3.9	94.7	9.35	119	11.3	128
M-LF	4.8	96.5	13.3	111	12.5	121
M-RR	8.3	91.9	17.3	118	18.3	140
M-YR	1.4	98.2	11.1	108	10.6	125
M-LR	4.8	104.5	11.9	105	11.4	122

Recoveries study in river water

The presence of organic substances in environmental aqueous samples can make analytical method less sensitive and inefficient because of the matrix effects and ion suppression effect. To validate this method in matrix conditions, the accuracy and precision of this method was assessed by analyzing water samples of different sources spiked with 5 μg/L standards. For filtered untreated river water spiked with 5 μg/L standards, RSD ranged from 6.21% to 17.3%, with a median of 11.9%. Spiking recoveries ranged from 105% to 135%.

Recoveries study in wastewater

Wastewater from domestic and industrial sources contains rich organic compounds, which accelerate the growth of algae. That's why a recoveries test was conducted in wastewater samples to ensure this LC-MS/MS method is validated. For filtered treated wastewater spiked with 5 μg/L standards, RSD ranged from 9.9% to 18.3%, with a median of 11.9%. Spiking recoveries ranged from 115% to 140% which were on the same level with those in untreated river water. Recoveries results from river and wastewater was also shown in Table 4.

Occurrence of cyanotoxins in drinking water treatment plants

A total of 68 water samples were collected from a variety of water resources, including Missouri river, Mississippi River, lake water, reservoir water, and underground wells in Missouri. Both untreated source and treated source water samples from each water treatment plant were analyzed. In all samples cyanotoxins of interests were all

detected below limit of quantification. These results were expected, because these were little algal blooms in state of Missouri. For CYN and STX, there was no occurrence reported in Missouri. Since no cyanotoxins were detected, 1 μg/L spiked samples were used to calculate recoveries, the recoveries range from 88% to 112% for treated source water and 77% to 135% for untreated source water.

Conclusions

A fast and easy LC-MS/MS method for determination of cyanotoxins in water samples has been described. The separation was carried out on a C-18 column with a gradient buffers. The analyte was detected by tandem mass spectrometry in positive ion mode. MRM experiments were used to monitor the ions of the analyte. The method limit of quantification ranges of 0.002-0.1 μg/L and a good linearity was obtained over a concentration range of 0.02-100 μg/L. The method has been successfully applied to different water matrix including reagent water, river water and wastewater in a reproducible manner. The quantitative analyses show a precision with RSDs of around 6% to 17% in untreated river water and 9.9% to 18.3% in treated wastewater. It also screened 68 water samples, both untreated source water and treated, from 34 different water treatment plants cross Missouri for cyanotoxins. Samples were collected from several water resources, including the Missouri river, the Mississippi River, groundwater, lakes, reservoirs, and wells. However cyanotoxins were all detected below limit of quantification in all samples.

Acknowledgments

We thank the Department of Chemistry and the Environmental Research Center at the Missouri University of Science and Technology, and Missouri Department of Natural Resource for financial support

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5. Occurrence and removal of *N*-nitrosamines by powered activated carbon in drinking water treatment plants

Abstract

The presence of *N*-nitrosamines in water bodies used either for drinking water or recreational purposes may present serious health risks for humans and pose a new technical challenge for water utilities when present in hazardous concentrations. In this work, a fast and sensitive method was developed for quantitative analysis of sub-ng/L levels of *N*-Nitrosamines in drinking and source waters using solid phase extraction followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) detection . Method detection limits (MDL) for the nine N-nitrosamines studied ranged from 0.1 to 15 ng/L. This newly developed method has been applied to N-nitrosamines analysis of untreated source water and disinfected drinking water in various Missouri water treatment systems. Nitrosodimethylamine (NDMA) and *N*-nitrosodi-*n*-butylamine (NDBA) concentrations ranged from nondetectable (below MDLs) to 16.7ng/L in the studied waters. The other nitrosamines were below the MDLs. The efficiency of removing *N*-nitrosamines from water using powered activated carbon was also studied at different pH values in both reagent water and natural water. It was found that *N*-nitrosamines were removed from 36-61% and 57-74% with 4 and 10 mg/L, respectively,with a common bituminous coal-based powdered activated carbon (PAC) with dosages from 4 to 10 mg/L, though removal at higher pH (i.e. 9.4) was reduced. Lignite coal- and wood-based PAC were much less effective at removing the suite of

Nnitrosamines studied. These results have significant implications for drinking water treatment.

Keywords

N-nitrosamines Powered activated carbon removal

1.1 Introduction

N-Nitrosamines are potent mutagenic and carcinogenic compounds in both humans and other animals (EPA, 1993). Their existence has been confirmed in food products, cosmetic products, tobacco smoke, soil, and ground water. In recent years, *N*-nitrosamnines, mainly nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), have also been found to form as water disinfection byproducts (Pehlivanoglu-Mantas *et al.*, 2006; Richardson, 2009). The carcinogenic potencies of these nitrosamines are considerably greater than those of trihalomethanes(Mitch *et al.*, 2003). The United States Environmental Protection Agency (USEPA) Integrated Risk Information System has classified these *N*-nitrosamines into the B2 group indicating probable carcinogenicity to humans. In addition to NDMA, the USEPA has listed five other nitrosamines, including NDEA, *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosodi-*n*-butylamine (NDBA), and *N*-nitrosopiperidine (NPIP), in the Unregulated Contaminant Monitoring Rule 2 (UCMR 2) to be monitored from 2008 to 2010 (EPA, 2006).According to the USEPA, while there is no current maximum

contaminant level (MCL), the maximum advisable concentration of these compounds in drinking water is 7 ng/L of NDMA, and 2 ng/L of NDEA, with a risk estimation of 10^{-5} (EPA, 1993; EPA, 2006)..

Water treatment via chlorination, chloramination, and chlorine dioxide of organic nitrogen-containing wastewater can produce NDMA at potentially harmful levels (Mitch *et al.*, 2003; Sedlak David *et al.*, 2005). NDMA can also form, or be leached, during treatment of water using anion exchange resins. Waters coming from disinfected sewage may contain more than 100 ng/L of NDMA (Asami *et al.*, 2009; Krauss *et al.*, 2009). Furthermore, the concentration of NDMA has been reported to reach 10 ng/L in surface waters and 20 ng/L in drinking water production wells that are under the influence of recharge water from wastewater treatment plants. While many nitrosamines are potentially formed as disinfection byproducts, only limited studies have addressed the formation and occurrence of a large suite of potential N-nitrosamines.

Drinking water disinfection with monochloramine (or free chlorine) can results in the formation of nitrosamines, including NDMA. The concentration of nitrosoamines increase with the concentration of monochloramine as does the reaction time (Mitch *et al.*, 2003). The maximum concentration of NDMA has been shown to be formed at pH 7-8, typical of many drinking water treatment plants (Zhao *et al.*, 2006). More recently, NPIP, N-nitrosopyrrolidine (NPYR), and N-nitrosodiphenylamine (NDPhA) have also been detected in drinking water in Canada (Zhao *et al.*, 2006). In Missouri, most of the drinking water systems are treated with chlorine and/or monochloramine, the oxidants that can form NDMA and other N-nitrosamines disinfection byproducts (DBP).

Existing methods to detect N-nitrosamines in drinking water are based on solid-phase extraction (SPE) for preconcentration and analysis by gas chromatography-mass spectrometry (GC-MS) (Charrois Jeffrey *et al.*, 2004; EPA, 2004) or liquid chromatography-mass spectrometry (LC-MS) (Zhao *et al.*, 2006).The most common way to remove N-nitrosamines in drinking water treatment is through ultraviolet photolysis, membrane treatment (e.g., reverse osmosis), and ozonation(Plumlee *et al.*, 2008; Sharpless Charles *et al.*, 2003; Sharpless *et al.*, 2003). Activated carbon adsorptive removal of nitrosamines is an alternative and promising method because it is often used in water treatment, potentially cost effective, and relative straightforward to apply or retrofit in water treatment systems (Dai *et al.*, 2009; Fleming *et al.*, 1996; Plumlee *et al.*, 2008; Sharpless *et al.*, 2003; Steinle-Darling *et al.*, 2007). Until now, the adsorption of N-nitrosamines by activated carbon in aqueous solution has not been systematically studied.

The objective of the present study was to develop a fast and accurate SPE-LC/MS/MS method to analyze nine N-nitrosamines in drinking water at environmentally relevant concentrations. The method developed herein combines the extraction efficiency of SPE with the high selectivity of LC/MS/MS detection. The method was applied to water samples from four different water treatment plants across Missouri (USA) to examine the concentrations and distribution of the nine nitrosamines. Finally, to assess the efficiency of removing N-nitrosamines by powered activated carbons (PACs), kinetic adsorption studies were conducted with various types of PAC. The effects of PAC dosage, contact time, and pH on adsorptive capacities was examined in both lab laboratory water and natural water.

1.2 Experimental

1.2.1 Chemicals and reagents

All chemicals and reagents used in this study were analytical grade or better unless otherwise stated. NDMA(N-nitrosodimethylamine), NDEA(N-nitrosodiethylamine), NDPA(N-nitrosodi-*n*-propylamine), NDBA(N-nitrosodi-*n*-butylamine), NPIP(N-nitrosopiperidine), and NDPHA(N-nitrosodiphenylamine) were obtained from Supelco (Bellefonte, PA, USA); NMEA(N-nitrosomethylethylamine) and NMOR(N-nitrosomorpholine) from Ultra Scientific (North Kingstown, RI, USA) and NDMA-d6 (N-nitrosodimethylamine-d6) from Isothopes Inc (Quebec, Canada) was used as internal standard(IS). Stock solutions and solutions of other concentrations were prepared by dissolving standards in Milli-Q water which was produced with a Millipore Elix 3 water purification system (Millipore, Bedford, MA). Three activated carbons were studied: WPH (Calgon Carbon Corporation), HydroDarco B (HDB, NORIT Americas Inc.), and Aqua Nuchar (AN, Meadwestvaco Corporation). Supelclean coconut charcoal SPE cartridges were from Supelco (St Louis, MO, USA)

1.2.2 Sampling collection

Water samples were collected in precleaned amber glass bottles from various drinking water treatment plants in Missouri (USA) by a method similar with previously used (Cheng *et al.*, 2010). For tap water collection, the aerator was first removed (if present) and then the water faucet was opened to allow the water flowed for about 5 min. The sample bottles were then filled to just overflowing to ensure no headspace in the

bottle. River water was collected with no headspace in large precleaned widemouth bottles. The filled water bottles were sealed and placed in cold cooler for overnight transfer to the lab. The water samples were filtered through a 0.45-μm nylon membrane filter and then were stored in a refrigerator until analysis. The analysis was completed within a week from the water collection.

1.2.3 LC-MS/MS detection of *N*-nitrosamines

Table 1 lists the N-nitrosamines studied along with the minimum reporting level indicated for each by U.S. Environmental Protection Agency (USEPA). Analysis of N-nitrosamines was performed using a triple-quadrupole mass spectrometer (API 4000Q TRAP) equipped with an Agilent 1100 series LC system. The mass spectrometer was operated in multiple reaction monitoring (MRM) transition mode at optimized parameters for each transition in positive ion mode. The analytical column was a 50x2.0 mm Phenomenex Gemini 3-μm C18 column. Separation was achieved by a gradient program with Eluent A (Milli-Q water with 3 mm ammonium acetate) and Eluent B (methanol with 3 mm ammonium acetate): Start from 10% B; increased to 45% B over 4min and increased to 100% B in 1min; maintained at 100% B for 5mins, then decreased to 10% B over 0.1min and maintained for 5 mins. The total run time was 15min. The total flow rate was 0.25 mL/min and the injection volume was 10 μl. Table 1 lists instrumental conditions and method parameters.

Table 1. Instrumental mass spectrometer conditions for study compounds.

MS parameter	NDMA	NDEA	NMEA	NDPA	NDBA	Npip	NMor	Npyr	NDPhA	IS
CAS#	62-75-9	55-18-5	0595-95-6	21-64-7	924-16-3	100-75-4	59-89-2	930-55-2	86-30-5	
MRL* (µg/L)	0.005	0.002	0.003	0.007	0.004	N/A	N/A	0.002	N/A	
Ion pair (m/z)	75/43	103/75	89/61	131/89	159/103	115/69	117/87	101/55	199/169	81/46
Collision gas ($l \cdot h^{-1}$)	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Curtain gas ($l \cdot h^{-1}$)	25	25	25	25	25	25	25	25	25	25
Ion spay voltage (V)	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000
Heater Temperature (°C)	450	450	450	450	450	450	450	450	450	450
Declustering potential (V)	56	56	51	36	41	46	56	36	61	51
Collision Cell Exit potential (V	6	12	10	4	8	12	14	8	12	6
Entrance potential (V)	10	10	10	10	10	10	10	10	10	10
Collision energy (V)	25	17	17	15	23	23	19	25	17	25

1.2.4 Solid phase extraction

Analytes were extracted using prepacked coconut-charcoal cartridges (Supelco MO USA). Each 400-mL water sample was prefiltered using a 0.45-um nylon filters (Whatman NJ USA), and then passed through the preconditionsed SPE cartridge at a flow rate of 3 mL/min under a slight vacuum. Elution was by 6 mL acetonitrile, followed by 3 mL acetone, and then 3 mL acetonitrile at a rate of 3 mL/min under low vacuum. The extracts were concentrated to a final volume of 400 µL under a nitrogen stream at temperature 25°C, followed by addition of 930 µL of laboratory Milli-Q water buffered with 3 mM ammonium acetate. The resulting concentration factor was approximately of 300 times. Extracts were then analyzed immediately via LC/MS/MS.

1.2.5 PACs adsorption experiments

Adsorption experiments were conducted to study the efficiency of removing N-nitrosamines with various dosages (i.e., 0, 1, 2, 4, and 10 mg/L) of PACs, pH levels (i.e., 5.4, 7.4, and 9.4), and adsorption times (i.e., 0, 0.5, 1, 2, 4, 8, and 24 hours). To

investigate the effects of competition with natural organic matters, both lab laboratory water and Missouri river water were used in the adsorption experiments.

Three activated carbons were studied: Calgon WPH, Norit HDB and AquaNuchar AN. Each PAC was dried in an oven at 105°C overnight prior to use. A stock suspension solution of 250 mg/L PAC was prepared by stirring the PAC into laboratory water for at least 30 minutes. The PAC stock was spiked to the desired dosage into 10 mL water samples containing 30 μg/L of a N-nitrosamine in 12-mL glass vials. The vials were quickly placed in LABQUAKE tumblers, and tumbled continuously at 8 RPM in a temperature controlled chamber at 20°C in the dark. 1.5-mL aliquots of each sample was then taken from each vial at specified times and centrifuges at 1000 rpm for 5 minutes to remove the PAC. The clear supernatant was then transferred into LC autosampler vials.

1.2.6 Total organic carbon detection and isoelectric point determination of PAC

Total organic carbon (TOC) concentrations of Missouri River water were measured using a TOC-5000A Total Organic Carbon Analyzer (Shimadzu, Columbia, MD, USA) by following manufacture'sinstruction. The isoelectric point (or zero point of charge, ZPC) was determined for each carbon using a Zetasizer Nano ZS90 (Malvern Instruments, Inc., Southborough, MA, USA).

1.3 Results and Discussions

1.3.1 LC/MS/MS optimization

A total nine N-nitrosamines were detected using LC/MS/MS within elutions ranging from 1.1 to 9.5 min. Figure 1 shows a representative MRM-mode LC/MS/MS chromatogram of the N-nitrosamines standards in laboratory water. NPYR and NMEA were the most difficult to be separate at high resolution, while ensuring that the method continues to work for other analytes.

However, the two co-eluting compounds were differentiated by different MRM ion pair transitions. All other N-nitrosamines were well separated chromatographically, and all peaks showed very good symmetry.

The precursor ion detected was the $[M+H]^+$ ion for all N-nitrosamines and the internal standard (IS) (NDMA-D6). The most abundant transition was used for quantitation based on individual optimizations. Calibration and quantification were performed on the basis of analyte/IS area ratio versus concentrations. The concentration of the NDMA-D6 IS used was 10 μg/L.

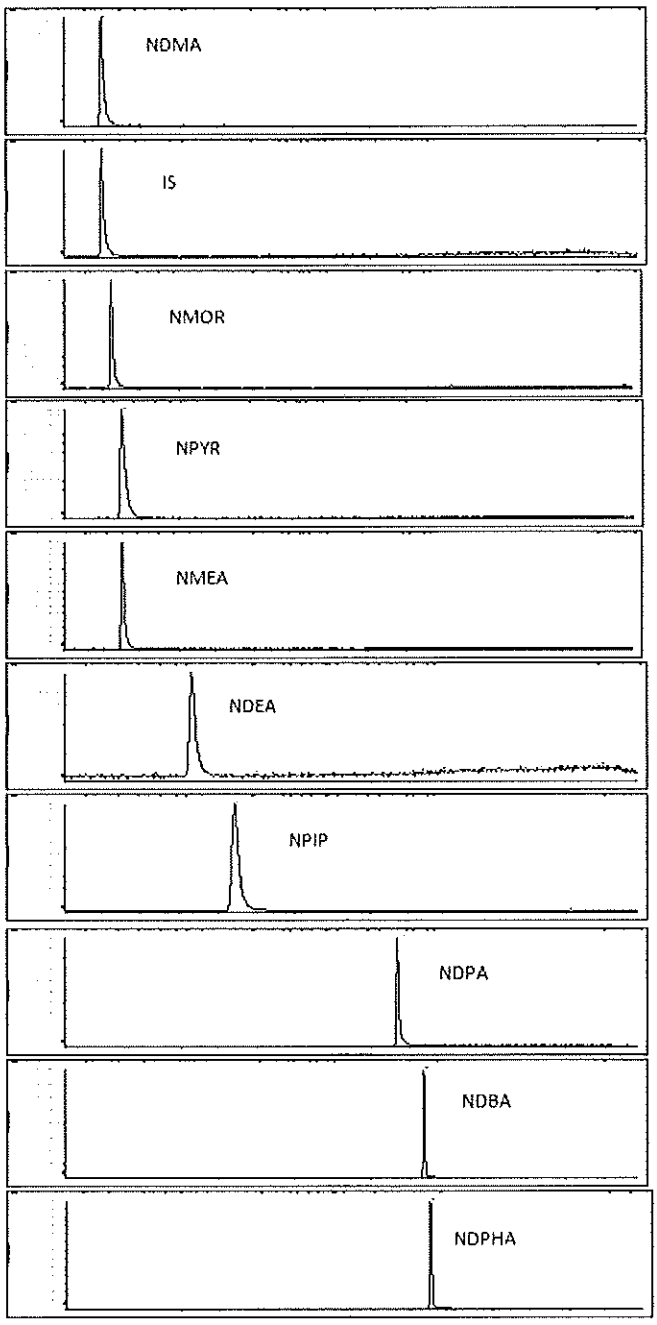

Figure 1. MRM LC/MS/MS chromatogram of *N*-nitrosamines standards in reagent water

The instrumental limit of detection (LOD) for each N-nitrosamine was determined as the lowest injected standard that gave a signal-to-noise (S/N) ratio between 3 and 5 calculated by measuring the signal peak height to averaged background noise ratio (per

Wisconsin Department of Natural Resources Laboratory Certification Program, April 1996). The background noise was based on the peak-to-peak baseline near the analyte peak. The method LODs for the study N-nitrosamines were between 0.01 and 2.5 µg/L in laboratory water. The precision of the LC/MS/MS method was evaluated by determining the relative standard deviation (RSD) of spiked samplesobtained from multiple (n=3) replicate analyses. For analyte-free laboratory water spiked with 0.5, 5, and 20 µg/L N-nitrosamines standard, respectively, RSD ranged from 1.22% to 19.2% (Table 2). For filtered untreated natural water spiked with 5 or 50 µg/L N-nitrosamines, the resulting RSDs ranged from 0.6% to 16.3%. A six-point standard calibration curve, at concentration ranges of 0.1-200 µg/L, exhibited good linearity (Table 2).

Table 2. Instrumental LC/MS/MS method validation results

	Instrumental			0.5 µg/L in reagent water		5 µg/L in reagent water		20 µg/L in reagent water		5 µg/L in raw water		50 µg/L in raw water	
Compound	LOD (µg/L)	Calibration Range (µg/L)	Linearity (R^2)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
NDMA	2	5-300	0.9998	N/A	N/A	27	109	4.24	104	N/A	N/A	1.09	98.7
NMEA	0.5	2.5-300	0.9999	N/A	N/A	4.74	104	1.91	102	6.08	94.1	0.66	102
NDEA	2.5	5-300	0.9997	N/A	N/A	N/A	N/A	4.79	94	N/A	N/A	0.45	101
NDPA	0.25	1-300	0.9999	19.2	82.3	2.37	103	2.98	97	6.46	105	0.99	99.5
NDBA	0.05	0.1-300	0.9952	3.9	96.1	2.99	96.7	2.86	103	1.35	101	0.72	102
Npip	0.5	2.5-300	0.9999	N/A	N/A	1.8	99.3	1.72	98.3	13.3	111	1.69	99.6
Nmor	0.5	2.5-300	0.9999	N/A	N/A	4.45	97.7	3.27	99.5	10.9	113	0.6	101
Npyr	0.25	1-300	0.9994	15.7	122	3.48	96.6	2.04	98.1	16.3	117	0.97	101
NDPhA	0.01	0.1-50	0.9994	3.19	99	1.86	99.8	1.22	98.7	1.13	101	0.92	101

1.3.2 SPE optimization

The method LOD (including solid phase extraction) was also determined for each N-nitrosamine using the same S/N methodology, but on samples processed through the entire SPE-LC/MS/MS method. The LOD was determined to range from 0.1 to 15 µg/L in laboratory water, and from 0.12 to 20 µg/L in natural water.

Prepacked coconut-charcoal cartridges were used to extract the N-nitrosamines from water as described above. To validate the optimized N-nitrosamines SPE method, recovery tests were performed by extracting and analyzing 400 mL laboratory water and prefiltered natural water spiked with different concentrations of N-nitrosamines. The recoveries were obtained from duplicate analyses of laboratory water spiked with 30 or 75 ng/L of a N-nitrosamines standard, spiked recoveries ranged from 28% to 120%. For natural waters spiked with 30 or 75 ng/L of a N-nitrosamine, spiked recoveries ranged from 26.4% to 133%. Table 3 shows the recovery data using optimized SPE procedures.

Table 3. Limits of detection (LOD) and recoveries in spiked reagent and source water

		Spiked reagent water				Spiked source water			
		Spiking 30 (ng/L)		Spiking 75 (ng/L)		Spiking 30 (ng/L)		Spiking 75 (ng/L)	
Compound	MDL (ng/L)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
NDMA	10	104	4.4	88	5.1	99	6.1	75	10.6
NMEA	2.5	81	8.7	79	3.7	98	0.9	67	3.1
NDEA	15	77	3.4	68	1.7	75	2.8	63	7.2
NDPA	1	81	1.2	87	3.0	89	0.6	76	3.2
NDBA	0.25	82	8.4	96	3.0	95	2.6	111	8.1
Npip	2.5	29	5.7	44	2.2	37	8.6	28	8.0
Nmor	2.5	91	0.8	98	1.8	99	11.5	90	2.5
Npyr	2	95	1.8	95	3.8	101	6.3	91	2.4
NDPhA	0.1	77	10.6	108	20.8	109	36.6	87	18.3

1.3.3 Occurrence of *N*-nitrosamines in water treatment plants

In this research, 14 water samples from four different water treatment plants in the state of Missouri were analyzed for N-nitrosamines including untreated source water, treated source water, treated water in distribution at an average residence time, and treated water in distribution at maximum residence time. Table 4 shows the concentrations of N-nitrosamines detected in water samples. In all samples, only NDMA and NDBA were detected at concentrations above their MDLs (Table 4). No other N-nitrosamines were all detected above their MDL. In the water treatment plant in which N-nitrosamines were detected, chloramines were then only disinfectant used. In the other water treatment plants, free chlorine was added initially for disinfection, followed by ammonia later in the train to form for chloramines for the residual disinfectant.

Table 4. *N*-nitrosamine concentrations detected in water samples

		Concentration (ng/L)								
Plant	Water type	NDMA	NDEA	NMEA	NDPA	NDBA	Npip	NMor	Npyr	NDPhA
1	Raw	nd	nd	nd	nd	0.28	nd	nd	nd	nd
1	Finished	16.7	nd	nd	nd	0.38	nd	nd	nd	nd
1	Regular distance	13.6	nd	nd	nd	nd	nd	nd	nd	nd
1	Max distance	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	Raw	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	Finished	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	Regular distance	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	Max distance	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	Raw	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	Finished	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	Regular distance	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	Max distance	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	Raw	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	Regular distance	nd	nd	nd	nd	nd	nd	nd	nd	nd
	MDL (ng/L) =	10	15	2.5	1.0	0.25	2.5	2.5	2.0	0.1

1.3.4 Removal efficiency in lab reagent water systems

Three different types of common PACs were examined in this study: (bituminous coal-based WPH, lignite coal-based HDB, and wood-based AN), Bituminous coal-based carbons (e.g., WPH) tend to have much smaller total surface areas, and an intermediate mix of macro- and micro-pores compared with wood-based and lignite-coal-based carbons(Jain *et al.*, 2004).Wood-based carbons (e.g., AN) tend to have a greater surface area, and a macroporous nature. Lignite-coal-based carbons (e.g., HDB) tend to have less total surface area and a highly microporous nature. The pH of zero charge for WPH, HDB and AN was measured at 2.0, 6.3 and 1.1, respectively.

Tests were first conducted in laboratory water at three different pH levels: 5.4, 7.4, and 9.4 at dosages ranging from 1 to 10mg/L and at a contact time of 4 hours (typical of many water treatment plants).The results showed that the sorption capacity for N-nitrosamines for the three PACs was WPH > AN > HDB in the laboratory water at all three pH levels.

For AN, pH had little effect on the removal efficiency of N-nitrosamines, where, specifically, there was no significant difference between sorption at the various pH levels. For AN, less than 20% removal was observed for all N-nitrosamines (except for NPIP and NDPHA) at typical PAC dosages of 1 to2mg/L at the common 4 hours contact time. At 10 mg/L PAC dosage, more than 40% removal was achieved for NDEA, NDBA, NPIP and NDPHA but not for the other N-nitrosamines.

Similar adsorption results were obtained with HDB. Specifically, there was no significant difference in adsorption results among various pH levels at typical PAC dosages of 1 to 2mg/L at the common4 hours contact time, only removals of NPIP and

NDPHA were observed to 40%. At the high dosage of 10 mg/L, more than 50% of NDBA and NDPHA were removed, including mor e than 95% of NDPHA. The amount of NDMA and NMOR removed by HDB was less than 15% for all pH levels, even for PAC dosages of 10 mg/L.For WPH, the efficiency of removing N-nitrosamines was poor at pH 9.4 compared with pH levels of 5.4 and 7.4 (Table 5). More than 40% of all N-nitrosamines were removed at a dosage of 2 mg/L of WPH at pH 5.4 and 7.4. With a dosage of 10 mg/L, more than 95% removal of NDMA, NDEA, NDPA, NPIP, and NDPHA, and more than 70% removal of others, was observed at pH 5.4 and 7.4.

Table 5. Removal of *N*-nitrosamines for three PACs in laboratory and natural waters. Removals are averages for pH 5.4, 7.4 and 9.4 (except for WPH in which 9.4 is significantly different (α=0.05), and reported separately)

		Mean removal (%)								
PAC Type and Dosage	pH	NDMA	NMEA	NDEA	NDPA	NDBA	Npip	Nmor	Npyr	NDPhA
Laboratory water										
WPH (1 mg/L)	Mean of 5.4/7.4	30	5	54	23	17	32	36	21	45
WPH (4 mg/L)	Mean of 5.4/7.4	70	67	96	64	60	68	51	56	75
WPH (10 mg/L)	Mean of 5.4/7.4	94	78	98	98	83	96	72	83	91
WPH (1 mg/L)	9.4	8	7	31	10	8	23	6	18	43
WPH (4 mg/L)	9.4	21	28	47	38	33	45	14	34	58
WPH (10 mg/L)	9.4	38	49	66	63	66	56	24	42	89
HDB (1 mg/L)	Mean of 5.4/7.4/9.4	3	6	21	5	3	34	4	18	45
HDB (4 mg/L)	Mean of 5.4/7.4/9.4	12	19	28	11	19	38	7	20	77
HDB (10 mg/L)	Mean of 5.4/7.4/9.4	16	30	39	22	62	41	9	22	96
AN (1 mg/L)	Mean of 5.4/7.4/9.4	3	11	20	6	8	32	2	18	26
AN (4 mg/L)	Mean of 5.4/7.4/9.4	22	25	43	28	28	40	6	22	37
AN (10 mg/L)	Mean of 5.4/7.4/9.4	32	36	46	63	63	50	12	26	52
Natural water										
WPH (1 mg/L)	Mean of 5.4/7.4	23	5	41	21	17	22	21	21	41
WPH (4 mg/L)	Mean of 5.4/7.4	45	56	61	34	44	53	36	46	55
WPH (10 mg/L)	Mean of 5.4/7.4	69	67	74	62	57	60	57	65	61
WPH (1 mg/L)	9.4	5	7	25	8	8	17	6	18	27
WPH (4 mg/L)	9.4	21	28	38	24	26	35	14	34	48
WPH (10 mg/L)	9.4	38	49	66	44	47	45	22	39	68
HDB (1 mg/L)	Mean of 5.4/7.4/9.4	2	6	17	6	3	22	4	10	35
HDB (4 mg/L)	Mean of 5.4/7.4/9.4	12	22	24	16	19	30	7	20	57
HDB (10 mg/L)	Mean of 5.4/7.4/9.4	13	32	30	28	51	34	10	25	73
AN (1 mg/L)	Mean of 5.4/7.4/9.4	3	11	11	6	9	16	2	12	16
AN (4 mg/L)	Mean of 5.4/7.4/9.4	16	24	29	16	21	31	6	22	25
AN (10 mg/L)	Mean of 5.4/7.4/9.4	25	29	35	24	36	40	8	24	38

Additional kinetic experiments were conducted to investigate the performance of WPH at varied contact times.. In these experiments, PAC dosages were applied and tested at contact times of 0.5, 1, 2, 4, 8,10 hours in laboratory water at pH 7.4. The results showed that more than 90% of all N-nitrosamines was removed at a dosage of 10 mg/L at contact time of 24 hours (Figure 2).

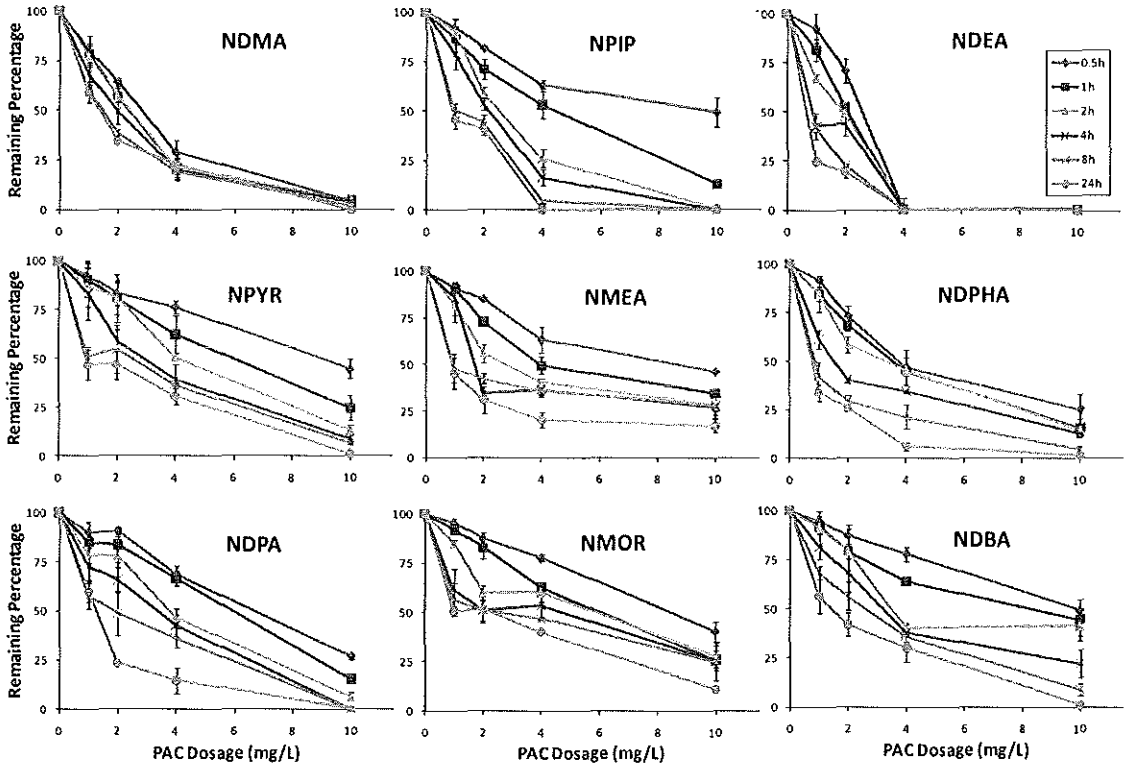

Figure 2. Removal of *N*-nitrosamines using WPH at different contact times at pH 7.4 in laboratory water

1.3.5 Removal efficiency in natural water systems

In this work, the effects on adsorption efficiency of competition with natural organic matters were studied using prefiltered Missouri River collected in March, 2010.

The water pH was adjusted phosphate buffer. The (DOC) concentration of the water was 10.2±1.8 mg/L as C (or at an intermediate level for a drinking water source).

For AN and HDB, the results were similar to the results observed using of laboratory water suggesting that compounds in the river did not significantly affect the adsorption of N-nitrosamines (Table 5). As observed in laboratory water for AN and HDB, pH had no significant effect on the amount of N-nitrosamines removed in natural water.

For WPH, the results were similar to those observed in laboratory water at pH 9.4 (Table 5). At pH levels of 5.4 and 7.4, however, much less adsorption was achieved in natural water, or about 60% of NDEA, NDPA, NPIP, and NDPHA versus more than 90% in laboratory water. While no pH-dependent trend was observed; the absence of a trend was not obvious because the difference between a pH of 9.4 and a low pH was smaller than that observed when using laboratory water. Due to the wide range of natural organic matters types and concentrations, the effects of NOM may vary significantly depending on the water source.

1.4 Conclusions

In this study, a fast and accurate method for quantitative analysis of N-nitrosamines using SPE-LS/MS/MS was developed with MDLs ranging from 0.1 to 15 ng/L. Results from four water treatment plants using monochloramines (as well as free chlorine in three plants) showed occurrence of only NDMA and NDBA in one sample. No other N-nitrosamines were observed.

WPH was the most effective PAC at removing most N-nitrosamines at typical dosages and contact times, though a pH-dependent trend was observed with lesser removal at high pH. NOM in natural waters had little effect on decreasing the sorption of the N-nitrosamines. AN and HDB demonstrated relatively low adsorptive capacity for the studied N-nitrosamines at all pHs levels even dosages of up to 10 mg/L in both laboratory and natural water. These results have significant implications for drinking water treatment. Specifically, depending on pH and the type and dosage of PAC used in a water treatment plant, vastly different removals of N-nitrosamines may be removed.

1.5 Acknowledgments

We thank the Department of Chemistry and the Environmental Research Center at the Missouri University of Science and Technology, and Missouri Department of Natural Resource for financial support.

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